

AUSTRALIAN AQUATIC VETERINARY EMERGENCY PLAN

AQUAVETPLAN

Disease Strategy

Crayfish plague

Version 1.0, 2005

AQUAVETPLAN is a series of technical response plans that describe the proposed Australian approach to aquatic animal disease incursions. The documents provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency-management plans.

Primary Industries Ministerial Council

This disease strategy forms part of:

AQUAVETPLAN Edition 2

This strategy will be reviewed regularly. Suggestions and recommendations for amendments should be forwarded to:

AQUAVETPLAN Coordinator
Aquatic Animal Health
Office of the Chief Veterinary Officer
Department of Agriculture, Fisheries and Forestry
GPO Box 858, Canberra ACT 2601
Tel: (02) 6272 4328; Fax: (02) 6273 5237
email: aah@daff.gov.au

Approved citation: Department of Agriculture, Fisheries and Forestry (2005). Disease strategy: Crayfish plague (Version 1.0). In: *Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN)*, Edition 2, Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, ACT.

Publication record:

Edition 2: 2005
Version 1.0, June 2005

AQUAVETPLAN is available on the internet at:
<http://www.affa.gov.au/aquavetplan>

© Commonwealth of Australia and each of its states and territories, 2005

ISBN 0-9752347-5-7

This work is copyright and, apart from any use as permitted under the *Copyright Act 1968*, no part may be reproduced without written permission from the publishers, the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF), acting on behalf of the Primary Industries Ministerial Council. Requests and inquiries concerning reproduction and rights should be addressed to the AQUAVETPLAN Coordinator (see above).

The publishers give no warranty that the information contained in *AQUAVETPLAN* is correct or complete and shall not be liable for any loss howsoever caused, whether due to negligence or other circumstances, arising from use of or reliance on this code.

IMPORTANT NOTE: Important regulatory information is contained in the OIE *International Aquatic Animal Health Code (OIE 2004)* for crayfish plague, which is updated annually and is available on the internet at the OIE website:

http://www.oie.int/eng/normes/fcode/fcode2004/en_acode.htm

Further details are given in Appendix 1 of this manual.

DISEASE WATCH HOTLINES

These telephone numbers connect callers to the relevant state or territory officer to report concerns about any potential emergency disease situation. Anyone suspecting an emergency disease outbreak should use this number for immediate advice and assistance.

New South Wales	1800 043 536	Northern Territory	1800 720 002
Queensland	07 3830 8550	Victoria	136 186
South Australia	1800 065 522	Western Australia	1800 815 507
Tasmania	1800 005 555		

Preface

This disease strategy for the control and eradication of crayfish plague is an integral part of the **Australian Aquatic Veterinary Emergency Plan**, or **AQUAVETPLAN (Edition 2)**.

The strategy sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of crayfish plague in Australia. The strategy was approved by:

- the National Aquatic Animal Health Technical Working Group of the Aquatic Animal Health Committee, at meeting 04 in May 2004;
- the Aquatic Animal Health Committee of the Primary Industries Standing Committee, at meeting 04 in June 2004; and
- the Primary Industries Standing Committee, at meeting 08 in March 2005.

Crayfish plague is listed by the OIE (World Organisation for Animal Health, formerly Office International des Epizooties) in the *International Aquatic Animal Health Code* (OIE 2004).¹

Detailed instructions for the field implementation of AQUAVETPLAN are contained in the disease strategies, operational procedures manuals and management manual. Industry-specific information is given in the **Enterprise Manual**. The full list of AQUAVETPLAN manuals that may need to be accessed in an emergency is shown below:

Disease strategies

Individual strategies for each disease

Operational procedures manuals

Disposal

Destruction

Enterprise Manual

Includes sections on:

- open systems

- semi-open systems

- semi-closed systems

- closed systems

Management manual

Control centres management

Aquatic Animal Diseases Significant to Australia: Identification Field Guide by Alistair Herfort, Department of Agriculture, Fisheries and Forestry, Canberra (Herfort 2004) is a source for some of the information about the aetiology, diagnosis and epidemiology of the disease and should be read in conjunction with this strategy.

This manual was drafted by Dr Frances Stephens, with the assistance of Ms Nicky Buller, Dr David Alderman (UK) and Drs Andrew Cameron, Mehdi Doroudi, Preston Suijendorp, Marty Deveney and Rachel Bowater.

Scientific editing: Biotext Pty Ltd, Canberra.

¹ See http://www.oie.int/eng/normes/fcode/a_index.htm (Accessed 11 May 2005).

This manual was adapted from similar manuals in AUSVETPLAN, the Australian emergency plan for terrestrial animal diseases, and from the AQUAVETPLAN **Enterprise Manual**. The format and content have been kept as similar as possible to those documents to enable animal health professionals trained in AUSVETPLAN procedures to work efficiently with this document in the event of an aquatic veterinary emergency. The work of the AUSVETPLAN writing teams and the permission to use the original AUSVETPLAN documents is gratefully acknowledged.

The text was amended at various stages of the consultation/approval process, and the policies expressed in this version do not necessarily reflect the views of all the members of the writing group. Contributions made by others not mentioned above are also gratefully acknowledged.

The revised manual has been reviewed and approved by the following representatives of government and industry:

Government	Industry
Commonwealth of Australia	Marron Growers Association of Western Australia
State of New South Wales	CSIRO Division of Livestock Industries
State of Queensland	Tasmanian Salmonid Growers' Association
State of South Australia	Tuna Boat Operators Association
State of Tasmania	Pearl Producers' Association
State of Victoria	Australian Prawn Farmers Association
State of Western Australia	Pet Industry Joint Advisory Council
Northern Territory	RecFish Australia
Australian Capital Territory	National Aquaculture Council

The complete series of AQUAVETPLAN documents is available on the internet at: <http://www.affa.gov.au/AQUAVETPLAN> (Accessed 11 May 2005).

Contents

Preface.....	3
1 Nature of the disease	9
1.1 Aetiology.....	9
1.2 Susceptible species.....	9
1.3 World distribution and occurrence in Australia	11
1.4 Diagnostic criteria.....	12
1.4.1 Clinical signs.....	13
1.4.2 Pathology	13
1.4.3 Laboratory tests.....	15
1.4.4 Differential diagnosis	15
1.5 Resistance and immunity	16
1.6 Epidemiology	17
1.6.1 Sources of <i>Aphanomyces astaci</i>	17
1.6.2 Reservoirs.....	17
1.6.3 Predisposing factors.....	18
1.6.4 Modes of transmission	20
1.7 Manner and likelihood of introduction to Australia	21
2 Principles of control and eradication.....	23
2.1 Introduction.....	23
2.1.1 International experience.....	23
2.1.2 Australian crayfish production systems	24
2.2 Methods to prevent spread and eliminate pathogens	25
2.2.1 Quarantine and movement controls.....	25
2.2.2 Tracing.....	26
2.2.3 Surveillance.....	26
2.2.4 Destruction of crayfish	26
2.2.5 Treatment of crayfish products and byproducts	27
2.2.6 Disposal of crayfish.....	27
2.2.7 Decontamination.....	27
2.2.8 Vector control	28
2.2.9 Sentinel animals and restocking	29
2.2.10 Public awareness	29
2.3 Feasibility of specific options for control in Australia.....	29
2.3.1 Eradication	29
2.3.2 Containment, control and zoning.....	30
2.3.3 Emergency harvesting	31
2.3.4 Trade, industry and environmental considerations.....	32

3	Policy and rationale	33
3.1	Overall policy.....	33
3.2	Overview of response options.....	34
3.2.1	Option 1 – Eradication	34
3.2.2	Option 2 – Containment, control and zoning.....	34
3.3	Strategies for control and eradication	34
3.3.1	Epidemiological investigations.....	34
3.3.2	Quarantine and movement controls	35
3.3.3	Zoning.....	35
3.3.4	Destruction of clinically diseased crayfish.....	35
3.3.5	Treatment of crayfish products and byproducts.....	35
3.3.6	Disposal.....	35
3.3.7	Decontamination	36
3.3.8	Surveillance	36
3.3.9	Tracing	36
3.3.10	Sentinel and restocking measures.....	36
3.4	Social and economic effects	36
3.5	Criteria for proof of freedom.....	37
3.6	Funding and compensation	37
Appendix 1	OIE <i>International Animal Health Code and Manual of Diagnostic Tests for Aquatic Animals</i>	39
Appendix 2	Overview of crayfish and the crayfish industry	41
Appendix 3	Common and scientific names of crustacean species mentioned in text	45
Appendix 4	Diagnosis of <i>Aphanomyces astaci</i>	47
Glossary	49
Abbreviations	55
References	57
Index	63

Tables

Table 1 Susceptibility of species of freshwater crayfish to crayfish plague 10

Table 2 Species found not to be susceptible to *Aphanomyces astaci* infection
in experimental studies..... 11

Table 3 Factors predisposing crayfish to acute crayfish plague infection..... 19

Figures

Figure 1 World distribution of crayfish plague 12

Figure 2 Crayfish plague in a susceptible species of crayfish..... 14

Figure 3 Establishment of declared areas to control crayfish plague 25

Figure 4 Distribution of crayfish species and *Aphanomyces astaci* 42

Figure 5 Phylogenetic relationships of the crayfish 43

1 Nature of the disease

Crayfish plague is a fungal disease that has the potential to cause large-scale mortality of freshwater crayfish in Australia. At present, the disease does not occur in Australia. However, it is important that state and territory governments and the red-claw, yabby and marron aquaculture industries are adequately prepared to manage a disease outbreak, because an incursion of the disease could devastate the freshwater crayfish aquaculture industry as well as wild populations of crayfish.

1.1 Aetiology

The aetiological agent of crayfish plague is the oomycete, *Aphanomyces astaci* Schikora (Schikora 1906).

Oomycetes (commonly called water moulds) are not considered to be 'true fungi' taxonomically, but have been placed in the phylum Oomycota. Within this phylum is the family Saprolegniaceae, which consists of the *Achlya*, *Aphanomyces* and *Saprolegnia* genera, with some species being pathogens of crustaceans, fish and plants.

A. astaci is a branching, non-septate fungus that produces spores under conditions that are favourable for the particular substrain. The spores can survive in fresh water for a variable time depending on water temperature and chemistry. Motile zoospores measuring 8–15 µm emerge from spores and attach to new hosts in the water body.

1.2 Susceptible species

Freshwater crayfish species from Australia, New Guinea, Japan and Europe are highly susceptible to crayfish plague; species from North America are more resistant but can die from the disease if their immune systems are stressed, such as by overcrowding or extreme weather (Roy 1993; Unestam 1969b, 1972, 1975).

The disease has not been reported in aquatic animals other than freshwater crayfish, but the Chinese mitten crab, *Eriocheir chinensis*, was susceptible to infection with *A. astaci* in an experimental study (Benisch 1940).

Of the commercial crayfish species in Australia, the red claw crayfish (*Cherax quadricarinatus*) and the yabby (*Cherax destructor*) have been tested and are susceptible to the disease, but there are no published reports of the susceptibility of marron (*Cherax tenuimanus*).

Only a few Australian species of crayfish have been experimentally challenged with crayfish plague, but it is safe to assume that all Australian freshwater crayfish may be highly susceptible to infection.

The susceptibility of many freshwater decapods to infection with *A. astaci* is unknown. Consequently, the likelihood of animals such as freshwater crabs and shrimp in Australia becoming carriers or developing clinical disease following infection with *A. astaci* in the wild is also unknown.

Table 1 details the susceptibility of crayfish species; Table 2 lists other species that are not experimentally susceptible.

Figure 4 in Appendix 2 illustrates the original geographical distribution of the three families of freshwater crayfish and the distribution of *A. astaci*. Figure 5 in Appendix 2 shows the phylogenetic relationship of various susceptible and resistant species of aquatic invertebrates that have been tested for susceptibility to crayfish plague.

Table 1 Susceptibility of species of freshwater crayfish to crayfish plague

Species name			
Scientific	Common	Disease severity	Region of origin
Non-Australian species			
<i>Pacifastacus leniusculus</i>	Signal crayfish	Resistant carrier ^{abf}	North America
<i>Procambarus clarkii</i>	Red swamp crayfish	Resistant carrier ^b	North America
<i>Orconectes limosus</i>	Mud crayfish	Resistant carrier ^b	North America
<i>Astacus astacus</i>	Noble crayfish	Overt disease ^{abdf}	Northwestern Europe
<i>Austropotamobius pallipes</i>	White-clawed crayfish	Overt disease ^b	Western and southwestern Europe
<i>Austropotamobius torrentium</i>	Stone crayfish	Overt disease ^e	Mountains in southwestern Europe
<i>Astacus leptodactylus</i>	Slender clawed or Turkish crayfish	Overt disease ^b	Eastern Europe, Middle East
<i>Cambaroides japonicus</i>		Overt disease ^b	Japan
<i>Cherax papuanus</i>		Overt disease ^f	Papua New Guinea
Australian species			
Smooth crayfish			
<i>Cherax destructor</i>	Yabby	Overt disease ^f	Australia
<i>Cherax quinquecarinatus</i>	Gilgie	Overt disease ^f	Western Australia
<i>Cherax quadricarinatus</i>	Red claw crayfish	Overt disease ^c	Queensland, Northern Territory
Spiny crayfish			
<i>Astacopsis gouldi</i>	Giant crayfish	Overt disease ^f	Tasmania
<i>Astacopsis fluviatilis</i>		Overt disease ^f	Tasmania
<i>Euastacus kershawi</i>		Overt disease ^f	Victoria
<i>Euastacus clydensis</i>		Overt disease ^f	New South Wales

Sources:

^a Unestam (1972)

^b Unestam (1969b)

^c Roy (1993)

^d Schikora (1906)

^e Vorburger and Ribí (1999)

^f Unestam (1975)

Table 2 Species found not to be susceptible to *Aphanomyces astaci* infection in experimental studies

Species name		
Scientific name	Common name	Classification
<i>Mysis relicta</i> ^a	Mysis	Subphylum: Crustacea, Order: Mysidacea
<i>Daphnia hyalina</i> ^b	Daphnia	Subphylum: Crustacea, Class: Branchiopoda
<i>Leptodora hyalina</i> ^b		Subphylum: Crustacea, Class: Branchiopoda
<i>Chydorus sphaericus</i> ^b		Subphylum: Crustacea, Class: Branchiopoda
<i>Bytotrephes longimanus</i> ^b		Subphylum: Crustacea, Class: Branchiopoda
<i>Cyclops strenuus</i> ^b	Copepod	Subphylum: Crustacea, Class: Copepoda
<i>Mesocyclops leuckarti</i> ^b	Copepod	Subphylum: Crustacea, Class: Copepoda
<i>Asplanchna priodonta</i> ^b	Rotifer	Phylum: Rotifera
<i>Bosmina</i> sp ^b		

Sources:

^a Unestam (1972) ^b Unestam (1969b)

1.3 World distribution and occurrence in Australia

Crayfish plague is a serious disease of freshwater crayfish in Europe. It is endemic in North America but rarely causes overt disease in North American species except when they are stressed (Smith and Söderhäll 1986).

Early reports of crayfish deaths suggest that crayfish plague was present in Europe by 1875 and, although the source of infection was never proved, it was suspected to have been introduced with live, imported North American crayfish. There were further introductions of North American crayfish to Europe, most notably in the 1970s (Nylund et al 1993). The earliest report in 1875 is from Italy, but the disease appears to have spread to the rest of Europe after 1975 from outbreaks in France and Germany. It has now been reported in Norway, Finland, Sweden, Russia, Germany, France, Switzerland, Spain, Greece, Turkey, the United Kingdom and Ireland. Losses of native European freshwater crayfish species have been catastrophic in infected waterways, and considerable resources have been allocated in several countries (including Finland and the United Kingdom) in an attempt to eradicate or control the disease.

The advent of molecular techniques has confirmed earlier suspicions that *A. astaci* entered Europe from North America. Four major strains of *A. astaci* have been found. Three have been identified on the signal crayfish, *Pacifastacus leniusculus*, which is one of the main North American species introduced to Europe. One strain appeared to have been introduced to Europe in the nineteenth century, a second was introduced to Sweden from the United States after 1970, and a third was found on *Pacifastacus leniusculus* from Canada (Huang et al 1994, Diéguez-Uribeondo and Söderhäll 1999). A fourth strain, found on *Procambarus clarkii* (red swamp crayfish) in Spain and the United States (Diéguez-Uribeondo and Söderhäll 1993), is adapted to warmer water temperatures. The origin of outbreaks in Europe can now be traced using molecular tools (Lilley et al 1997, Oidtmann et al 1999).

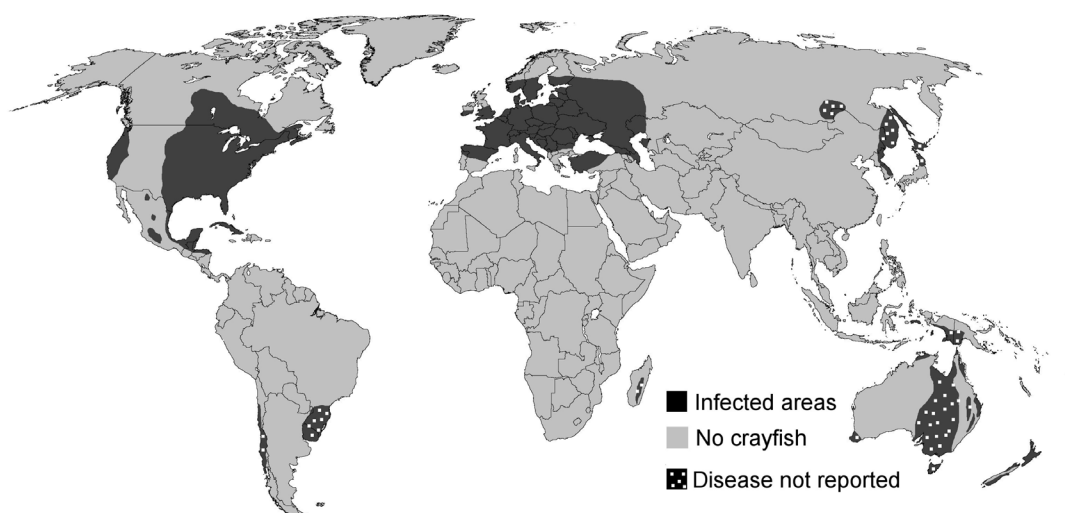


Figure 1 World distribution of crayfish plague

Note: The light grey areas indicate areas in which crayfish do not occur naturally. In some of these areas, non-native species have been introduced.

Crayfish plague has never been reported in Australia or found during passive surveillance. To date, no outbreaks of crayfish plague have been reported in red claw in Europe, Ecuador or the United States despite the export of this species (Westman and Westman 1992, Romero 1997). Likewise, although *Pacifastacus leniusculus* has been introduced to Japan, and *Procambarus clarkii* to Kenya, South America, China, Japan, Taiwan and the Philippines (Huner 2002, Lewis 2002), there have been no reports of crayfish plague in those countries.

1.4 Diagnostic criteria

Crayfish plague must be suspected whenever there is a mortality event in which many crayfish die but other aquatic animals remain unaffected. Diagnosis is based on clinical signs, histopathology and laboratory culture of the disease agent. Methodology for diagnosis and isolation techniques is based on culture and characterisation of the fungus, and can be found in:

- the OIE *Manual of Diagnostic Tests for Aquatic Animals* (OIE 2003);
- the OIE *International Aquatic Animal Health Code* (OIE 2004);
- Crayfish plague (*Aphanomyces astaci*) Australian and New Zealand Standard Diagnostic Procedure (ANZSDP)²; and
- Molecular diagnostic tests to detect epizootic ulcerative syndrome (*Aphanomyces invadans*) and crayfish plague (*Aphanomyces astaci*) (ANZSDP) (in preparation)³.

² See <http://www.daff.gov.au/content/publications.cfm?ObjectID=A5BC0B2B-90EA-46AA-8AA79501B4ED5ADC>- (Accessed 22 June 2005).

³ See www.scahls.org.au (Accessed 22 June 2005).

1.4.1 Clinical signs

Clinical signs of disease are often subtle, especially in acute outbreaks when environmental conditions, high zoospore numbers and a high density of susceptible crayfish results in large-scale mortality within days of infection. The behaviour of affected animals provides a clue to a diagnosis of crayfish plague (affected crayfish often demonstrate unusual gait or activity), but the clinical signs are not specific for this disease.

While laboratory tests and experiments that demonstrate the transmission of disease from infected to uninfected crayfish are necessary to confirm the diagnosis, infected animals may show some of the following characteristics and behaviours (Schäperclaus 1927, 1935; Nybelin 1931, 1936; Unestam and Weiss 1970; Unestam 1972; Alderman et al 1984, 1987, 1990; Nylund and Westman 1995a; Oidtmann et al 1999):

- high mortality;
- easy to catch and lethargic when close to death;
- apparent poor limb coordination;
- lie on their dorsal surface ('belly up');
- constant leg movements;
- loss of appendages;
- leave shelter during daylight hours;
- move from water to land during daylight hours;
- walking with stiffly stretched legs ('walking on stilts');
- paralysis;
- lost or weak tail 'flip' or 'flick' response;
- may have brown, yellowish or dull grey patches on the base of legs or underside of the abdomen; and
- fine, white fungal growth visible at affected sites on legs and abdomen soon before or after death.

1.4.2 Pathology

Gross lesions

Gross pathological signs are not specific for crayfish plague, and further tests must be undertaken to confirm the presence of *A. astaci*. Clinical signs vary greatly, ranging from no obvious signs or lesions to the presence of visible hyphae protruding from soft parts of the exoskeleton. Other signs of the disease are the presence of opaque, whitish flesh between abdominal segments or brown melanised spots on the walking legs and abdomen.



A: normal crayfish

B: infected crayfish

Figure 2 Crayfish plague in a susceptible species of crayfish

Note areas of melanisation at the base of the legs and whitening of the abdominal segments in photograph B. Melanin deposits may not always be obvious in infected crayfish. Crayfish plague can only be diagnosed by the use of laboratory techniques, and the appearance or behaviour of crayfish can be used only as a guide to possible presence of the disease. (Photographs © British Crown Copyright, courtesy Dr DJ Alderman)

Histopathology

Using a light microscope, *A. astaci* hyphae may be seen in excised soft cuticle from affected areas of the infected tissues.

The hyphae are 5–10 µm in diameter, aseptate and branching, and stain black against a green background using a Grocott–Gomori stain. Hyphae are seen on and beneath the exoskeleton, particularly in the soft cuticle of the joints, membranes and abdominal segments where the organism is able to penetrate the skeleton. Growth of hyphae tends to be restricted to the area of cuticle penetration, but they can also grow along the ventral nerve cord and brain ganglion. However, growth can be sparse and may not be seen on histological examination. Occasionally, hyphae are seen in the eye but rarely in other organs, and they do not invade the musculature until late in the infection. The tissue around the area of infection becomes necrotic and yellow-brown. Hyphae in the cuticle close to the epidermis and in the layer adjacent to the epicuticle can be surrounded by deposits of melanin and haemocytes. In the later stages of the disease, zoosporangia and zoospores form on the exoskeleton (Schäperclaus 1935, Nybelin 1936, Unestam and Weiss 1970).

1.4.3 Laboratory tests

The state/territory chief veterinary officer (CVO) must be notified immediately of any suspected incidence of crayfish plague.

Preliminary identification of *A. astaci* may be undertaken by some state/territory diagnostic laboratories. Upon suspicion of crayfish plague, state/territory governments will arrange for samples to be sent to the CSIRO Australian Animal Health Laboratory (CSIRO-AAHL), Geelong, for confirmatory diagnosis.

Submission of specimens

Freshly dead or dying crayfish showing signs of the disease should be submitted to the nearest state/territory diagnostic laboratory. Samples should be submitted to the AAHL Fish Diseases Laboratory via the diagnostic laboratory and the CVO. Successful culture of the pathogen is more likely if samples are transported to the laboratory within 12 hours of death of the animal.

Animals should be placed on ice but not frozen, as freezing will kill the oomycete. Dead animals can be put into a plastic bag and placed over ice in a small esky or other suitable container. The lid of the container should be secured with tape, and the outside wiped with a disinfectant such as sodium hypochlorite (100 ppm available chlorine) to prevent transmission of the infection. The fungus is susceptible to drying, so transmission of viable spores is a low risk factor.

Sampling equipment may be available on site, or may be obtained from state/territory fisheries or agricultural officers (see the AQUAVETPLAN **Enterprise Manual** for contact details).

Laboratory diagnosis

Fungal culture

Confirmation of suspected crayfish plague is achieved by culturing for the presence of *A. astaci*, with the identity confirmed by sporulation test. Culture of the fungus may take up to 15 days, and overgrowth with bacterial flora and other fungi is also a problem. See Appendix 4 for further details of tests.

Molecular diagnosis

Molecular diagnostic tests are being developed for *A. astaci* (Fisheries Research and Development Corporation funded project 2001/621). These tests will offer a rapid detection method using fluorescent in situ hybridisation (FISH) on either tissue smears or histopathology slides, and provide a result within hours. A polymerase chain reaction test that will detect the fungus from fresh or preserved tissues or culture is also being developed.

1.4.4 Differential diagnosis

White patches of abdominal muscle on limb bases, the mid-abdomen and the perianal region may be caused by the microsporidian parasite *Thelohania cotejeani* (Schäperclaus 1927, Polglase and Alderman 1984), although muscle infected with *Thelohania* spp is a more vivid white than that infected with *A. astaci*.

Lesions caused by fungi such as *Fusarium solani* and brown, melanotic spots from previous injuries or other infections must be differentiated from crayfish plague, but rarely cause the high and rapid mortality of *A. astaci* infection (Schäperclaus 1927).

Sudden deaths of large numbers of freshwater crayfish can also result from environmental disturbances, some bacterial infections or toxicity (such as exposure to pesticides). However, these causes of mortality are likely to also affect other crustaceans in the habitat.

1.5 Resistance and immunity

Most freshwater crayfish species around the world are believed to be susceptible to infection by *A. astaci* (see Table 1). When highly susceptible species are infected, there is often 100% mortality with little or no evidence of acquired immunity (Unestam and Weiss 1970).

Despite many decades of infection, there are no reports of development of resistance to the disease in European species of crayfish (Unestam 1973, Westman 1991, Svärdson 1992). Susceptible animals die within several days or weeks of infection (Unestam 1969a, 1973), followed by complete eradication of crayfish from the watershed downstream from the site of infection.

North American freshwater crayfish species are carriers of the disease, but not every animal is infected. In carrier animals, the fungus is present in black or brown lesions in the soft cuticle, and is most often seen 3–4 months after moulting (Unestam 1969b, 1972; Unestam and Söderhäll 1977; Nylund and Westman 1983; Svärdson et al 1991).

European species sometimes have brown, melanised spots, as were seen in Turkey before large-scale mortality (Baran and Soylu 1989, Rahe and Soylu 1989, Skurdal and Taugbøl 2002). This may have been caused by epidemiological factors, such as zoospore numbers and water temperatures that resulted in a prolonged period between infection and death (Alderman et al 1987). Conversely, it may demonstrate some degree of innate resistance (Unestam 1969b).

The immune response of invertebrates differs from that of vertebrate animals, but does involve both humoral and cellular defence mechanisms. The major host defence against fungal infection involves melanisation of the fungal hyphae (Unestam and Weiss 1970). The formation of melanin is a result of activation of the prophenoloxidase (proPO) system. The proPO enzyme is produced in the cuticle and haemocytes following exposure to carbohydrates – the β -1,3-glucans in the fungal cell walls (Unestam 1972, Unestam and Söderhäll 1977, Söderhäll 1981, Söderhäll and Cerenius 1999). The glucans activate a serine protease which, in turn, induces a Ca^{++} -dependent phenoloxidase attachment to the fungal hyphae (Söderhäll 1981, Smith and Söderhäll 1986, Sritunyalucksana and Söderhäll 2000).

Species susceptibility to crayfish plague appears to be the result of differences in the immune response and the amount of chitinase or proteinase inhibitors present in the cuticle, rather than any structural differences between species (Nyhlén and Unestam 1975, Unestam and Söderhäll 1977, Cerenius et al 2003). Phenolic substances appear to be produced more rapidly around *A. astaci* hyphae in resistant species, preventing further spread into the underlying muscle and nerve cord (Unestam and Weiss 1970, Unestam and Söderhäll 1977).

1.6 Epidemiology

The pattern and severity of disease outbreaks depend on several factors. Host factors, such as the innate susceptibility of the species, the presence of stress factors and damage to the exoskeleton, are important in determining the severity of disease in individual animals and in populations (Cerenius et al 1988). Factors relating to the fungus, such as its substrain and optimal water temperature, influence the number of zoospores produced and, therefore, the potential for the disease to spread to susceptible crayfish. Other factors, such as stocking density, are also important in determining the pattern of an outbreak in a crayfish population.

1.6.1 Sources of *Aphanomyces astaci*

Outbreaks of crayfish plague often occur when *A. astaci* is introduced to previously unaffected populations of susceptible crayfish. Common sources of infection include:

- introduction of infected, live or dead crayfish to a water system;
- introduction of water from infected water bodies; and
- transfer by way of water droplets and zoospores from nearby water on vectors, which include boats, nets, boots, birds, fish and terrestrial animals.

1.6.2 Reservoirs

Animal reservoirs

Crayfish

Infected and dead crayfish are the main source of *A. astaci* zoospores in the environment. Frequently, the introduction of resistant carriers with no or few visible lesions is responsible for transfer of the disease to susceptible crayfish that inhabit the same water body (Vorburger and Ribi 1999). On other occasions, when crayfish and zoospore densities are low, a long-term, low-grade pattern of mortality may occur because there are fewer crayfish to act as reservoirs of infection (Alderman et al 1990, Diéguez-Uribeondo and Söderhäll 1993, Fürst 1995, Alderman 2002).

Other animals

Mammals, such as otters, mink or muskrats, and waterbirds have sometimes been blamed for spreading crayfish plague in Europe, but scientific studies have found that zoospores do not survive the temperatures of the gastrointestinal tract of mammals or birds (Oidtmann et al 2002). The movement of fish may be of greater concern in the spread of infection (Alderman et al 1987, Oidtmann et al 2002) because zoospores remain viable in fish mucus and fish intestinal tracts. The cleaning and gutting of fish from other water bodies is another potential source of infection (Häll and Unestam 1980).

Environmental reservoirs

Water

Under ideal conditions, even small amounts of water can transfer enough zoospores to infect a new water body. As few as 1.3 zoospores per millilitre of

water can infect susceptible animals (Alderman et al 1987). The zoospores rapidly spread downstream in river current; movement upstream is slower and occurs by the movement of infected crayfish. Weirs, or large tracts of water that contain no crayfish, act as barriers to the spread of the disease via carrier crayfish.

Equipment

Most new outbreaks in countries where crayfish are harvested recreationally or commercially are caused by human activity (Westman 1991), including translocation of contaminated water or crayfish from their site of origin and trapping using contaminated traps or nets that have not been adequately disinfected (Alderman et al 1987, Reynolds 1988, Nylund et al 1993).

1.6.3 Predisposing factors

North American species of crayfish appear to have a host-parasite balance that ensures continuity of both species without major population crashes. Overt disease resulting in crayfish mortality only occurs in North American species after stressful events, such as overcrowding or unseasonable weather (Smith and Söderhäll 1986, Diéguez-Uribeondo and Söderhäll 1993). This sporadic pattern of disease outbreaks is typical of long-term coexistence of host and parasite (Unestam 1973, Fürst 1995).

Geographical location rather than phylogenetic origin is important in determining susceptibility to crayfish plague. For example, although *Pacifastacus leniusculus* is in the same family as European crayfish, it is resistant to the disease, whereas *Cambaroides japonicus* from Japan is susceptible despite being phylogenetically related to crayfish from eastern United States (Unestam 1972).

Stress factors that predispose crayfish to crayfish plague include suboptimal water quality, high stocking density and intra- and interspecies aggression. The presence of abrasions on the exoskeleton also increases the likelihood of *A. astaci* gaining entry into the crayfish cuticle. This is more likely to occur immediately after moult, when the exoskeleton is still soft.

Table 3 lists known predisposing factors.

Endogenous factors

Species

Many studies have highlighted the difference between the susceptibility to *A. astaci* of Northern American crayfish and species from other parts of the world, including Australia (see Table 1). Fewer spores are needed to cause disease in susceptible species, and there is less evidence of host reaction to the fungus (Unestam 1969b, 1975; Unestam and Weiss 1970). Although North American species of crayfish are frequently infected with the fungus, it does not usually cause overt disease (Nyhlén and Unestam 1975, Unestam and Söderhäll 1977).

Stage of moult

Crayfish appear to be more susceptible to crayfish plague at the time of moulting (Smith and Söderhäll 1986). Although no scientific evidence has been presented to substantiate the influence of this factor, the physiological events during moulting and/or the increased ease of injury of the soft exoskeleton during this period may predispose crayfish to the disease.

Table 3 Factors predisposing crayfish to acute crayfish plague infection

Host	Pathogen	Environment
Susceptible species ^{afg}	Strain suitable to environment ^d	Salinity suitable to <i>A. astaci</i> ^e
Recent moult ^b	Number of zoospores produced ^{ae}	Suitable temperature for <i>A. astaci</i> ^{cde}
Damaged exoskeleton ^a		Poor water quality, causing stress to crayfish ^{hi}
High stocking density ^j		
Stress ^{bc}		
Starvation ^b		
Handling ^b		
Presence of other diseases ^b		

Sources:

^a Unestam and Weiss (1970)	^e Unestam (1969a)	ⁱ Fürst (1995)
^b Smith and Söderhäll (1986)	^f Vorburger and Ribí (1999)	^j Svårdson et al (1991)
^c Cerenius et al (1988)	^g Unestam (1969b)	
^d Diéguez-Urbeondo et al (1995)	^h Diéguez-Urbeondo et al (1993)	

Exogenous factors

Water temperature

A. astaci is a parasite adapted to living in or near chitin (Unestam 1969a). The substrains found on *Pacifastacus leniusculus* in Europe are inherently cool/temperate water pathogens. Crayfish are readily infected with the disease between 2°C and 20°C whereas, at 25°C, not all animals become infected (Unestam 1969a, Smith and Söderhäll 1986, Alderman et al 1987). At 13°C, zoospore production is greater than at 20°C, and zoospores are likely to be infective for longer (Cerenius et al 1988). At temperatures below 10°C, infected crayfish take longer to die, and there are more gross signs such as limb autotomy (limb loss) and melanisation (Alderman et al 1987). At higher temperatures and high challenge, gross muscle necrosis is often the only disease sign. In a study using Australian red claw (Roy 1993), *A. astaci* was more pathogenic at 14°C than at 20°C.

Characteristics of pathogen substrain

There are at least four strains of *A. astaci*, each having different growth, sporulation and zoospore characteristics and different temperature tolerances (Diéguez-Urbeondo and Söderhäll 1993, 1999; Huang et al 1994). The substrain found on *Procambarus clarkii* in Spain grows and sporulates better and has greater zoospore motility between 18°C and 25°C than other substrains (Diéguez-Urbeondo and Söderhäll 1993). This appears to be an adaptation of *P. clarkii* to Spain's climate, which is warmer than that of eastern United States. More strains with adaptations to different environmental factors will probably be identified in the future.

Re-emergence of zoospores

A. astaci zoospores can encyst and re-emerge as zoospores up to three times (Cerenius and Söderhäll 1985). This is thought to be an adaptation to a parasitic lifestyle by increasing the survival time of zoospores and their chance of finding a crayfish host. This process is temperature dependent. At 14°C, zoospores are unlikely to survive for more than one week (Cerenius and Söderhäll 1985), but survival may be longer at 2°C (Unestam 1969a). The pathogen survives for a limited time once infected crayfish have been removed from the water body, but it

is recommended that water bodies not be restocked with crayfish until three months after removal of the last crayfish (Kenneth Söderhäll, pers comm, cited by Alderman 2002).

Zoospore density

The densities of zoospores and crayfish influence the time taken for the disease to infect and kill individual animals and the rate of disease spread in a susceptible population (Unestam 1969a, Unestam and Weiss 1970, Diéguez-Uribeondo and Söderhäll 1993). Where zoospore density is high, water temperature is optimal for *A. astaci* and there are large numbers of susceptible crayfish, mortality occurs within days and entire populations may be rapidly eliminated. Rapid, large-scale mortalities will not occur in a watershed with a low density of native crayfish and low zoospore numbers, but it is very difficult to be sure that crayfish plague is not present (David Alderman, Senior Microbiologist, the Centre for Environment, Fisheries and Aquaculture Sciences, Weymouth, United Kingdom, pers comm, August 2003).

Salinity

Seawater and brackish water inhibit the release of zoospores from sporangia and zoospore motility (Unestam 1969a). However, neither the critical level of salinity nor the effect of concentrations of various mixtures of ions has been studied.

Physical damage to the exoskeleton

Damage to the exoskeleton is likely to increase the likelihood of infection by *A. astaci* (Unestam and Weiss 1970, Smith and Söderhäll 1986). Injury and damage is more likely to occur during handling, moulting or fighting (Vorburger and Ribi 1999).

1.6.4 Modes of transmission

The translocation of North American crayfish with benign infections is a major source of new disease epidemics in the United Kingdom. *Pacifastacus leniusculus* is almost always found in British watersheds experiencing epidemics of crayfish plague for the first time (Alderman 1993). It should be noted that not all North American crayfish carry the fungus, and uninfected North American and European crayfish coexist in some parts of Europe. However, the disease has been impossible to contain in many European countries, especially where water bodies are separated by short distances and where human activity levels are high.

Horizontal spread

A. astaci is transmitted horizontally in fresh water, causing acute disease in susceptible crayfish or chronic lesions of no clinical significance in resistant crayfish. Horizontal transmission can occur by:

- translocation of infected crayfish;
- water on wet boats, pumps, nets, traps or other equipment;
- sediment containing encysted zoospores, including on contaminated items such as damp, muddy boots; or
- vector-to-crayfish spread by fish, terrestrial animals and humans.

Vertical spread

Vertical transmission, in which disease is spread from one generation to the next by infected eggs, is not a mode of transmission for *A. astaci*.

1.7 Manner and likelihood of introduction to Australia

The two epidemiological weaknesses of *A. astaci* are its obligate parasite status, whereby zoospores must find a new crayfish host within days or perish, and the susceptibility of mycelia and spores to boiling, drying, freezing and chemicals. These characteristics may help in preventing entry of the pathogen into Australia. However, the disease agent could still be imported illegally in:

- live crayfish from overseas;
- uncooked crayfish products;
- untreated water that may or may not contain fish, plants or invertebrates; or
- fishing or boating equipment from infected areas, particularly if the equipment is still damp.

Water temperature is likely to affect the probability of *A. astaci* becoming established in Australia. Some substrains of the fungus prefer temperatures of about 13–20°C, and parts of Australia may be too warm for them to propagate. However, the substrain found in *Procambarus clarkii* in Spain (Diéguez-Uribeondo and Söderhäll 1999) sporulates at higher temperatures and would be likely to infect Australian crayfish.

2 Principles of control and eradication

2.1 Introduction

Just one zoospore or one drop of water can start an outbreak of crayfish plague. Control measures, sometimes backed by specific legislation such as the Prohibition of Keeping of Live Fish (Crayfish) Order 1996 (England and Wales), are in place in several European countries. Despite legislation and extensive public awareness campaigns (Westman and Westman 1992, Nylund and Westman 1995b, Oidtmann et al 1999), spread of the disease by human activity continues to be the major source of new outbreaks.

While small areas (one or a few lakes) have been cleared of crayfish plague after an outbreak, the disease has never been eradicated from larger infected areas with complex water bodies and river systems.

Eradication of the disease from an area or water body requires the complete removal of crayfish and thus the infectious agent. This is difficult to achieve, particularly when plague-resistant species are present in a watershed (Vorburger and Ribí 1999). Populations of susceptible crayfish can recover naturally following an outbreak if no infected crayfish or zoospores are present and if juveniles or adults migrate from uninfected areas. However, it is often more than a decade before stocks reach harvestable numbers, and new outbreaks often occur in recovered stocks when the disease is reintroduced from nearby water bodies.

2.1.1 International experience

Control measures in Europe (Cueller and Coll 1983, Skurdal and Taugbøl 1992, Westman 1992, Taugbøl and Skurdal 1993, Oidtmann et al 1999, CEFAS 2000) have included:

- prohibition of movement of live or dead crayfish between water bodies;
- prohibition of transport of crayfish other than in new boxes;
- drying and/or disinfection of boats and equipment before transfer to a second water body and between crayfish catching seasons;
- bans on the importation of second-hand harvesting or handling equipment;
- boiling of crayfish at their place of capture or by the first purchaser;
- bans on catching crayfish in infected waters;
- bans on the emptying of water in tankers or containers into a different water body; and
- bans on the importation of live or uncooked crayfish.

Rapid diagnosis and effective action after sudden mass deaths of crayfish is important in managing a potential outbreak of crayfish plague. Two problems identified during overseas outbreaks have been the use of expensive or unsuitable disinfection methods in the field and inappropriate or unclear instructions for crayfish farmers (Alderman 2002).

Australia has two advantages that may help in eradication of the disease: our dry environment and our hot climate, which may reduce spore production and the growth rate of mycelia. Nevertheless, eradication of the disease from rivers or water bodies in close proximity will be extremely difficult.

2.1.2 Australian crayfish production systems

In Australia, freshwater crayfish occur in three main aquatic systems (see the AQUAVETPLAN **Enterprise Manual** for a full description). In the event of an outbreak of crayfish plague, the methods of control or eradication will be determined in part by the type of system affected.

Open systems

If the outbreak occurs in an open system (a river or creek), eradication will be extremely difficult. Removal of all crayfish from the water body by chemical means, with consequential destruction of other aquatic animals, may be the only viable way to eradicate the infective agent, but this option is likely to be unpalatable to the public. A publicity campaign would be required in order to raise public awareness of the importance of eradication and to educate the public of the need to prevent spread of the pathogen.

A precedent for such a large-scale removal of fish exists. In Norway, the chemical rotenone was used to remove all fish from rivers in an attempt to eliminate the skin parasite *Gyrodactylus salaris*, which had become established in local salmon populations. This was successful in smaller rivers.

Semi-open and semi-closed systems

Many crayfish aquaculture facilities are semi-open systems with fenced ponds or dams, but run-off may enter waterways or ponds. In semi-closed systems, the movement of crayfish can be controlled and there is partial control of the distribution and flow of water.

Closed systems

In closed systems, such as reservoirs, farm dams or aquaculture facilities with no run-off into nearby waterways, both crayfish and water movements can be controlled. If an outbreak occurs in a closed system, eradication may be easier to achieve with less ecological damage.

2.2 Methods to prevent spread and eliminate pathogens

2.2.1 Quarantine and movement controls

Prevention of the movement of infected crayfish and the pathogen is the key priority in the event of an outbreak or suspected outbreak. Affected farms, water bodies and rivers must be identified and quarantined without delay.

The quarantine and movement restrictions that should be implemented immediately upon suspicion of crayfish plague are:

- establishment of specified areas (see Figure 3; see Section A of the AQUAVETPLAN **Enterprise Manual** for more details), which will include
 - an infected area or premises
 - a restricted area surrounding an infected premises or area
 - a control area, which is a buffer between the restricted area and free areas(together, these three areas form the *declared area*; the free area is the area outside the declared area and may include large areas of Australia in which *A. astaci* does not occur or remains unassessed);
- bans on the movement of live crayfish into, within or out of infected and restricted areas;
- suspension of recreational fishing in the declared area; and
- restrictions or bans on movements of people, vehicles or equipment within and between farms, dams or river systems containing crayfish in the declared area.

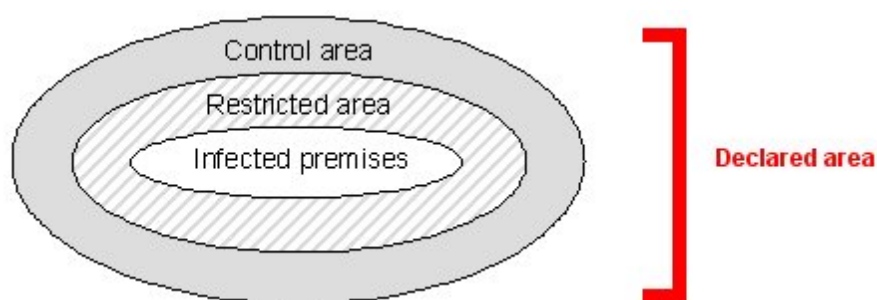


Figure 3 Establishment of declared areas to control crayfish plague

The implementation of bans and restrictions will be a dynamic process determined by the location and extent of the disease outbreak and whether the aim is to eradicate the disease or to control its spread. Some restrictions listed above may be deemed impractical or unnecessary in the particular circumstances, but others will be critically important to eradication or control.

Zoning

Zones may be gazetted. Zones are geographical regions that are delineated to decrease the spread of disease from an infected zone to a disease-free zone. The process of zoning is outlined in the **AQUAPLAN Zoning Policy Guidelines**⁴ and in the *OIE International Aquatic Animal Health Code* (OIE 2004). Zones are based on species distribution, the geographical and hydrological characteristics of water bodies and landforms, and predictions of the most likely method of spread of disease. Zoning might not be used in an outbreak.

2.2.2 Tracing

Tracing is used to discover the method and pattern of disease spread, and may include 'trace-forward' and 'trace-back'. It is crucial to defining and modifying the dimensions of the restricted area and requires investigations that determine:

- the initial source and location of infection;
- the movement of infected crayfish and water;
- the possible movement of vehicles, humans, animals and equipment that may act as vectors of the disease; and
- the existence and location of other potentially infected premises or areas.

2.2.3 Surveillance

Surveillance is used to detect new disease outbreaks, to define the infected area for quarantine and movement restriction purposes, and to monitor eradication and control programs. Three methods may be appropriate:

- a targeted survey of crayfish from different sites, using laboratory testing to determine their disease status;
- observation of crayfish behaviour and mortality in various crayfish habitats around the region, state or nation; and
- the use of healthy, susceptible crayfish as sentinel animals in water bodies with a high likelihood of having crayfish plague – if zoospores are present, susceptible crayfish are expected to develop overt disease.

2.2.4 Destruction of crayfish

An important method of preventing further spread of crayfish plague is to ensure that crayfish and zoospores are not moved from infected premises. In outbreaks of emergency diseases of terrestrial animals, slaughter is used to create a buffer zone around infected premises, and this method is also appropriate for crayfish plague. Methods suitable for the euthanasia of crayfish are outlined in more detail in the **AQUAVETPLAN Destruction Operational Procedures Manual**.

⁴ See <http://www.affa.gov.au/content/output.cfm?ObjectID=D2C48F86-BA1A-11A1-A2200060B0A00717> (Accessed 16 June 2005).

2.2.5 Treatment of crayfish products and byproducts

Crayfish from a declared area may be suitable for human consumption, but must be processed to destroy viable *A. astaci* mycelia or zoospores. Boiling for one minute kills all mycelia and spores, and therefore ensures that crayfish products are no longer infectious (CEFAS 2000, Oidtmann et al 2002), as does freezing to -20°C for 72 hours (Oidtmann et al 2002).

2.2.6 Disposal of crayfish

Destroyed crayfish must be disposed of by a method such as burning, or burial followed by liming (Cueller and Coll 1983). Methods suitable for the disposal of dead animals are outlined in more detail in the AQUAVETPLAN **Disposal Operational Procedures Manual**.

2.2.7 Decontamination

Several methods of decontamination can be used to decontaminate items such as boats, boots, nets, lamps, tools, baskets and containers.

Desiccation

Mycelia and spores are both killed by drying for 48 hours (Smith and Söderhäll 1986). Drying surfaces may be the most effective and easy method of preventing spread of the disease on crayfish handling equipment and boats in some circumstances.

High temperature

30°C for 30 hours or 37°C for 12 hours is sufficient to kill zoospores and mycelia (Smith and Söderhäll 1986, Oidtmann et al 2002), but temperatures of 50°C are preferred as some strains may survive the lower temperature (Professor K Söderhäll, Department of Comparative Physiology, Uppsala University, Sweden, pers comm, August 2003).

Chemical disinfection

Several chemicals successfully kill mycelia and zoospores. The most widely used and readily available is sodium hypochlorite, but iodophors, hydrogen peroxide and peracetic acid in hydrogen peroxide can also be used (Lilley and Inglis 1997). Gross contamination, such as mud on boots and equipment, must always be removed before disinfection.

- *Chlorine*. Sodium hypochlorite and many of the solid chemicals used to provide free available chlorine in swimming pools and spas in Australia, such as calcium hypochlorite, are suitable sources of chlorine. Chlorination of water used to clean equipment and effluent from infected premises or processing facilities renders them free of *A. astaci* before their release into the environment. It requires 100 ppm of free available chlorine for 30 seconds (Alderman and Polglase 1985). Because Liquid chlorine sources rapidly lose their strength, it is necessary to measure free chlorine immediately before use. Note that chlorine-based disinfectants damage rubber products, such as vehicle tyres and gumboots.

- *Iodine*. A concentration of 100 ppm available iodine supplied as iodophors can be an effective disinfectant for crayfish plague. The necessary exposure time depends on the brand of iodophor, and may be up to 32 minutes (Alderman and Polglase 1985). Iodophors are less corrosive than chlorine-based disinfectants but are not suitable for all items, as they leave residual stains (Alderman and Polglase 1985).
- *Peracetic acid*. Treating surfaces for 5 minutes with 100 ppm of 5% peracetic acid in hydrogen peroxide, a commonly used disinfectant in the food industry, has been shown to be an effective disinfectant (Lilley and Inglis 1997).

Some chemicals may have a place in the control of crayfish plague but cannot be recommended as disinfectants on the basis of current knowledge. Häll and Unestam (1980) noted that formaldehyde (formalin) inhibited hyphal growth, spore formation and germination of *A. astaci* at 80 mg/L formaldehyde. Formalin might be a useful disinfectant at higher concentrations, but there are no other published reports of its efficacy against *A. astaci*.

Malachite green is another such chemical. It was a commonly used fungicide in aquaculture, but its use is no longer condoned as it is a potential carcinogen and bioaccumulates in animal tissues (Treves-Brown 2000). After one hour, *A. astaci* mycelia and zoospores in 2 mg/L malachite green are no longer viable (Häll and Unestam 1980, Lilley and Inglis 1997).

Magnesium chloride has also been investigated for its effects on *A. astaci*. Low concentrations decreased mycelial growth and prevented sporulation. At higher levels, it prevented transmission of the disease to susceptible crayfish (Rantamäki et al 1992).

Environmental considerations

Effluent from processing plants and aquaculture farms that have handled potentially infected animals or been infected with *A. astaci* must be contained and disinfected to reduce the likelihood of spread of the disease. The potential negative environmental impact of water treatment during an outbreak must be considered and strategies must be implemented to reduce this impact. Chlorine and iodine-based disinfectants can be rendered harmless before discharge by the addition of sodium thiosulphate.

Burial sites for crayfish that have died or been destroyed during an outbreak must be selected to prevent run-off and seepage that could pose a threat to the environment.

2.2.8 Vector control

Controlling the spread of crayfish plague between watercourses is difficult because zoospores can be spread by water, by carrier crayfish walking to new locations, by other animals such as waterbirds or rats, or by translocation on equipment or motor vehicles. Understanding possible vectors of infection is important in selecting appropriate policies and controls during an outbreak.

2.2.9 Sentinel animals and restocking

Restocking should only occur after *A. astaci* has been eliminated from the water body. This may involve setting of traps to verify that no crayfish remain following the outbreak and the use of sentinel animals (disease-free, susceptible crayfish held in cages at different locations in the water body).

It can be difficult to determine that *A. astaci* is no longer present, as sentinel crayfish may not be exposed to zoospores if there are low population densities of infected crayfish and zoospores in a water body. Experience in Europe suggests that restocking should not occur within three months of elimination of the last infected crayfish, and that sentinels would need to be used for two years to demonstrate that the watershed contains no *A. astaci* (K Söderhäll, pers comm, cited by Alderman 2002).

Crayfish used for restocking must be disease free. Sometimes restocking occurs naturally following outbreaks in which all crayfish have been removed from a lake or downstream of a certain point. In such instances, uninfected crayfish migrate from uninfected lakes, streams or tributaries or from upstream of the area that had been infected with crayfish plague (Alderman 1993, Taugbøl and Skurdal 1993).

2.2.10 Public awareness

Publicity campaigns are important in outbreaks to raise public awareness of crayfish plague and to educate the public in order to prevent the spread of infection. In Europe, posters and pamphlets have been produced and awareness campaigns have been run on television in an attempt to achieve this.

2.3 Feasibility of specific options for control in Australia

The feasibility of controlling an outbreak of crayfish plague in Australia depends on the nature and location of the outbreak and the management strategy that is adopted. Essentially, there are two control options:

- *Eradication* – eradication or complete elimination of *A. astaci* from Australia (highest level of control measure and cost).
- *Containment, control and zoning* – limiting the fungus to areas with endemic infection, prevention of further spread and protection of uninfected areas.

Emergency harvesting of crayfish for human consumption may be possible under either option, but carries a high likelihood of further spreading infection and can jeopardise the success of an eradication strategy. See Section 2.3.3 for requirements for emergency harvesting.

2.3.1 Eradication

Eradication of crayfish plague requires the complete removal of *A. astaci*. This is achieved by removing all crayfish and preventing spread of the fungus, thereby preventing infection of uninfected crayfish populations. The disease agent cannot survive for more than a few weeks without its crayfish host.

Eradication is unlikely to be successful or feasible if epidemiological investigations determine that infection is widespread, has no point source, cannot be contained, or is present or potentially present in wild crayfish in rivers. The ability of *A. astaci*

to spread rapidly downstream, infecting wild crayfish populations, may make it impossible to eradicate in river systems. In Europe, eradication has been unsuccessful once infected carrier crayfish became established in rivers or lakes.

Unexposed crayfish

Immediate destruction of all crayfish within a restricted area will decrease the likelihood of spread of infection to uninfected crayfish. This is the preferred option, but it is not easy and is unlikely to be successful in many cases.

Uninfected crayfish may be emergency harvested for human consumption provided there is no likelihood of their exposure to *A. astaci*. Strict hygiene practices are required at processing, and on-farm processing may be preferable, as this will prevent any potential infection during transport to off-site processing plants.

Unexposed crayfish will only be allowed to grow out to market size if future exposure to infection can be prevented. Strict farm hygiene practices and transportation protocols are necessary to ensure that there is no transfer of infection to non-infected crayfish populations via crayfish, water, equipment or any husbandry practice.

Exposed or potentially exposed crayfish

All live crayfish within a restricted area must be assumed to be infected. Therefore, grow-out is not an eradication option, as it would increase the likelihood of spread of infection to other farms or wild crayfish stocks. All crayfish must be removed from the water, destroyed and disposed of safely as soon as possible to avoid further spread of infection to wild and farmed crayfish and the aquatic environment.

Although such crayfish are safe for human consumption, their emergency harvest may jeopardise the success of an eradication strategy if it is not carefully controlled to ensure that product, water and equipment leaving the processing facility do not contain viable *A. astaci*.

Infected crayfish

Diseased and dead crayfish are the main source of *A. astaci* in the environment, and successful eradication requires their urgent removal from the water body, destruction and safe disposal. Burial sites should be chosen carefully to ensure that there is no contact with waterways or vectors.

If any resistant carrier crayfish, such as North American species, are present in a water body, they must be removed if eradication is to be successful.

2.3.2 Containment, control and zoning

A zoning program and associated control measures to maintain uninfected zones will be necessary if the authorities decide to attempt to contain and control crayfish plague. The feasibility of this option will depend on the location of the crayfish and the likely reservoirs of infection.

Movement restrictions on potentially infected crayfish, crayfish products, water and equipment will be an important part of a zoning program. The potential for animal vectors, such as waterbirds and rats, to spread crayfish plague between water bodies will also need to be considered.

Unexposed crayfish

Aquaculture and harvesting of unexposed crayfish for human consumption can occur as normal. The method of harvest, the equipment used and the location chosen should ensure that there will be no exposure to infection. On-farm slaughter and processing may be preferable on uninfected sites, as this will prevent any potential infection during transport to an off-site processing plant. Water and vehicles are vectors of crayfish plague, and this route of transmission must be managed.

Immediate destruction is an option for unexposed crayfish populations in an infected zone, as it will decrease the chance of spread of infection to these stocks and prevent propagation of the disease.

Exposed or potentially exposed crayfish

Exposed and potentially infected crayfish must be treated as infected, with immediate destruction remaining an option.

In a declared area, grow-out and slaughter may be feasible without further spread of infection. However, final products must be processed to the degree required for the designated market; for example, if products are destined for domestic human consumption in areas free of *A. astaci*, they must be processed to remove or inactivate viable fungal hyphae and zoospores.

Movement restrictions on crayfish and crayfish products, processing equipment, people, vehicles and boats will be necessary to protect uninfected zones.

Infected crayfish

In a control and containment program, infected, dead and dying crayfish are handled in the same manner as they are during an eradication program (see Section 2.3.1).

2.3.3 Emergency harvesting

Emergency harvesting of crayfish for human consumption is most likely to be used for crayfish of marketable size in aquaculture facilities within declared areas.

Emergency harvesting carries a high likelihood of further spreading infection and can jeopardise the success of an eradication strategy. Strict control measures, approved by environmental protection and consumer protection agencies, are necessary to prevent further spread of infection, including the following:

- Processing should be on site to prevent spread of zoospores in water.
- Product removed from harvested sites or processing plants must be treated to prevent the spread of viable *A. astaci* mycelia and zoospores.

- All equipment and personnel involved in harvesting, slaughter and processing must be adequately disinfected.
- Quarantine restrictions and procedures must apply to the infected site, including personnel, equipment and vehicles.
- Effluent from slaughter and processing (including holding water and waste offal) must be treated to inactivate *A. astaci* mycelia and zoospores.

2.3.4 Trade, industry and environmental considerations

In most European countries, crayfish plague is endemic and has had a substantial detrimental effect on populations of native crayfish and professional crayfish harvesting industries. The disease has affected recreational fisheries in Sweden. In addition, *A. astaci* has been introduced to aquaculture facilities growing European species of crayfish, resulting in considerable economic loss to business operators. In some countries, native species have been largely replaced by the more plague-resistant and rapidly growing North American species of crayfish. Sometimes this has resulted in further spread of crayfish plague and habitat damage caused by the burrowing habits of these animals.

Trade regulations, market requirements and food safety standards must be considered as part of a control strategy. Permits may be required from the relevant authorities to allow products derived from declared areas to be released and sold for human consumption.

Export markets

Crayfish plague is listed as a reportable disease of aquatic animals by the OIE. Consequently, many countries require imports to be certified free from crayfish plague. Access of Australian crayfish to export markets may be seriously affected by an incursion of crayfish plague in Australia.

The Australian Quarantine and Inspection Service (AQIS) should be contacted for further information on current export market requirements.

Domestic markets

Public opinion and various government departments and agencies may determine the feasibility of releasing crayfish product from potentially infected animals onto the domestic market. Food safety requirements would need to be met and the spread of *A. astaci* to uninfected water or crayfish must be prevented.

3 Policy and rationale

3.1 Overall policy

Crayfish plague is a highly contagious fungal disease of freshwater crayfish that has the potential to cause almost 100% mortality in farmed and wild crayfish in Australia. The disease could devastate the natural ecology of freshwater habitats in affected areas because populations of native species of freshwater crayfish are likely to become seriously depleted. The freshwater crayfish aquaculture industry would also be seriously affected by the loss of overseas markets and increased costs from the implementation of extra disease control measures.

The methods used to control an outbreak of crayfish plague in Australia will depend on the nature of the outbreak. Following epidemiological investigation, the director of fisheries and/or the chief veterinary officer (CVO) of the state or territory in which the outbreak occurs will select the most suitable control option.

There are two possible control options for an outbreak of crayfish plague in Australia:

- ☞ *Option 1 – eradication of the disease agent, *Aphanomyces astaci*, from Australia; and*
- ☞ *Option 2 – containment, control and zoning with the aim of containing the fungus within known endemic areas and preventing its further spread to uninfected areas.*

Each of these control options involves the use of a combination of strategies, such as:

- ☞ *quarantine and movement controls on crayfish, crayfish products, water and equipment in declared areas to prevent spread of infection;*
- ☞ *destruction of crayfish that may be infected with *A. astaci* as soon as possible to prevent further production and spread of fungal zoospores;*
- ☞ *decontamination of facilities, crayfish products, water and equipment to eliminate the fungus;*
- ☞ *surveillance to determine the source and extent of infection and to provide proof of freedom from the disease; and*
- ☞ *zoning to define infected and maintain disease-free zones.*

Crayfish plague has the capacity to cause severe, long-term ecological damage to the freshwater environment, and production losses and loss of market access in the crayfish farming industry. It will therefore be necessary to act immediately to control or eradicate the disease.

The director of fisheries and/or the CVO of the state or territory in which the outbreak occurs will decide on the appropriate response option in consultation with the aquatic Consultative Committee on Emergency Animal Diseases (aqCCEAD). The decision will be made after consideration of results of the epidemiological investigation (see Section 3.3.1). While eradication may be the preferred option, it may not be feasible, given the limited success of eradication and control policies in Europe.

For a description of the notification arrangements, order of procedures, management structures and roles of personnel following suspicion of the presence of *A. astaci* in Australia, refer to the **AQUAVETPLAN Control Centres Management** manual.

3.2 Overview of response options

3.2.1 Option 1 — Eradication

If epidemiological investigations determine an obvious point source of infection that has been or may be contained with minimal or no spread of the fungus (eg in a closed system such as an aquarium or fully recirculating system), an eradication strategy may be successful and should be attempted. Eradication has the highest short-term economic costs, but if eradication succeeds the long-term economic and ecological benefits are likely to outweigh the costs.

Eradication is unlikely to be successful or feasible if epidemiological investigations determine that infection is widespread, if the source of infection cannot be determined, or if the disease is present or potentially present in wild crayfish.

Eradication procedures include those outlined in Sections 2.2 and 2.3.1 of this manual.

3.2.2 Option 2 — Containment, control and zoning

If the disease becomes established in wild crayfish, eradication is likely to be impossible. In this case, containment and prevention of further spread of disease is the preferred control option. The aim is to keep uninfected areas or zones free of crayfish plague. Restrictions on the movement of crayfish and crayfish products and ongoing surveillance and monitoring programs will be necessary to support a zoning program. The strategies outlined in Section 2.2 and 2.3.2 of this manual will be implemented.

3.3 Strategies for control and eradication

3.3.1 Epidemiological investigations

Thorough epidemiological investigation and tracing is fundamental to the success of eradication or zoning programs and must be conducted immediately upon suspicion of an outbreak of crayfish plague to determine the actual and potential spread of infection. This knowledge is required to determine the most scientifically and economically feasible response option.

3.3.2 Quarantine and movement controls

Quarantine and/or movement controls should be implemented on any material or equipment capable of transmitting infection. Restricted areas and control areas (see Section 2.2.1) should be established immediately, and their dimensions refined as further information becomes available from epidemiological investigations and hydrographical data. See the AQUAVETPLAN **Enterprise Manual** for information on different enterprise systems and response options.

For an eradication program, quarantine and movement controls must be stringently enforced on crayfish, crayfish products, water and any vectors in declared areas. Movement controls should be maintained until the disease is either eradicated or declared endemic.

For containment, control and zoning, movement controls are essential to maintain uninfected areas/zones. Restrictions must apply to removal from the infected area of anything capable of transmitting *A. astaci* from infected to uninfected crayfish, watersheds, dams, ponds, aquaculture facilities or processing plants. The routes of natural spread of *A. astaci* by animals and water should also be examined and managed as effectively as possible.

3.3.3 Zoning

If zoning is implemented, an active targeted surveillance program for *A. astaci* will be necessary within the uninfected zone.

3.3.4 Destruction of clinically diseased crayfish

Immediate removal, destruction and safe disposal of all diseased and dead crayfish are essential to the success of any response strategy. These crayfish are the main source of *A. astaci* hyphae in the environment. Refer to the AQUAVETPLAN **Destruction Operational Procedures Manual** for details of destruction methods.

3.3.5 Treatment of crayfish products and byproducts

Unexposed products may be marketed and disseminated with minimal likelihood of transmission of infection. However, products from crayfish populations exposed or potentially exposed to *A. astaci* will require processing and/or may have a restricted market in order to maintain areas free of crayfish plague.

The treatment of potentially infected crayfish products and byproducts must take into account trade regulations, market requirements, food safety standards and potential spread of the pathogen via product (see Section 2.2.5). If destined for human consumption, harvested crayfish can be stored in a freezer until a definitive diagnosis is obtained and decisions are made regarding release of product. This will prevent the spread of infection and allow salvage of product for sale (provided the relevant authority approves release).

3.3.6 Disposal

Immediate, safe disposal of all infected crayfish, wastes and effluent, and of equipment that cannot be decontaminated, is necessary for the eradication of the fungus. Burial sites should be chosen carefully to ensure that there is no contact with waterways or vectors. See Section 2.2.6 of this manual and the AQUAVETPLAN operational procedures manuals for details.

Effluent must be treated to prevent spread of infection.

3.3.7 Decontamination

Eradication

All buildings, tanks, materials and equipment that could be contaminated, including nets, boats and vehicles, must be cleaned and disinfected. If disinfection cannot be achieved effectively and quickly, then contaminated materials, equipment and buildings should be destroyed or other arrangements made to prevent the spread of *A. astaci*. At all stages of decontamination, steps must be taken to prevent the spread of infection via water, wastes or materials, especially into natural waterways.

Containment, control and zoning

Thorough cleaning and disinfection of water and equipment that may move from an infected to a disease-free zone, including nets, boats and vehicles, is important.

3.3.8 Surveillance

Active surveillance for the presence of *A. astaci* in restricted and control areas should continue until crayfish plague is declared either eradicated or endemic. If a zoning program is implemented, targeted active surveillance for *A. astaci* outside the restricted and control areas will be necessary to support the declaration of zones free of crayfish plague.

3.3.9 Tracing

The location of *A. astaci* spores and mycelia will be determined by tracing the possible movement of infected material, such as carrier crayfish, susceptible live and dead crayfish, water, aquaculture and harvesting equipment, motor vehicles, boats, and effluent and waste from processing plants. Trace-back determines the possible source of infection by tracing the movement of these things before the disease outbreak. Trace-forward determines the potential for spread of infection from the location of the outbreak.

3.3.10 Sentinel and restocking measures

Caged sentinel crayfish can be used only after the site has been thoroughly decontaminated. Sentinel animals may be useful to ascertain freedom from infection in water bodies or watersheds where sparse populations of infected crayfish could still remain.

Large-scale restocking with susceptible species should only occur once the water body is known to be free from the disease.

3.4 Social and economic effects

If an outbreak of crayfish plague were to occur in Australia, the cost to the crayfish industry and the environment would be enormous. Not only would there be loss of farmed and wild stock, but the cost of control and eradication campaigns would be substantial. If the disease were not eradicated, *A. astaci* in the environment would continue to be a source of infection, causing new outbreaks. Additionally, the export of many commodities that might harbour *A. astaci* hyphae or zoospores would be adversely affected.

3.5 Criteria for proof of freedom

Wherever possible, proof of freedom should comply with the international standards that apply at the time, such as those outlined in the OIE *Manual of Diagnostic Tests for Aquatic Animals* (OIE 2003).

3.6 Funding and compensation

There are currently no cost-sharing arrangements in place for aquatic animal diseases.

Appendix 1 *OIE International Animal Health Code and Manual of Diagnostic Tests for Aquatic Animals*

OIE Aquatic Code

The objective of the OIE (2004) *International Aquatic Animal Health Code* (the OIE Aquatic Code) is to prevent the spread of aquatic animal diseases, while facilitating international trade in fish and fish products. This annually updated volume is a reference document for use by veterinary departments, import/export services, epidemiologists and all those involved in international trade.

The current edition of the OIE Aquatic Code (7th edition) was published in 2004 and is available on the OIE website at:

http://www.oie.int/eng/normes/fcode/a_index.htm
(Accessed on 11 May 2005)

The following chapter is relevant to this manual:

Chapter 4.1.7 Crayfish plague

OIE Aquatic Manual

The purpose of the OIE (2003) *Manual of Diagnostic Tests for Aquatic Animals* (the OIE Aquatic Manual) is to contribute to the international harmonisation of methods for the surveillance and control of the most important aquatic animal diseases. Standards are described for laboratory diagnostic tests and the production and control of biological products (principally vaccines) for veterinary use across the globe.

The current edition of the OIE Aquatic Manual (4th edition) was published in 2003 and is available on the OIE website at:

http://www.oie.int/eng/normes/fmanual/A_summry.htm
(Accessed on 11 May 2005)

The following chapter is relevant to this manual:

Chapter 4.1.7 Crayfish plague

OIE Disease Technical Cards

The purpose of the OIE *Disease Technical Cards* is to provide a summary of information relevant to the disease, its characteristics, diagnosis and control.

The current *Disease Technical Cards* are available on the OIE website at:

http://www.oie.int/aac/eng/cards/en_diseasecard.htm
(Accessed on 11 May 2005)

The following card is relevant to this manual:

Crayfish plague

Further information

Further information about the OIE Aquatic Code and Aquatic Manual is available on the OIE website at:

http://www.oie.int/eng/normes/en_acode.htm
(Accessed on 11 May 2005)

Appendix 2 Overview of crayfish and the crayfish industry

Crayfish, crabs, lobsters, prawns and shrimp are all decapods (order Decapoda). The freshwater crayfish belong to two superfamilies. The Parastacoidea are found only in the southern hemisphere (in Australia, New Guinea, South America and Madagascar). Crayfish from the northern hemisphere are members of the superfamily Astacoidea, in which there are two families. Crayfish from the eastern parts of the United States and from Japan are members of family Cambaridae, whereas European species and *Pacifastacus leniusculus* from the west of the United States are in the family Astacidae.

Freshwater crayfish do not occur naturally in continental Africa, and in Asia they occur naturally only in Turkey, Iran, Japan and a small area of mainland China.

Figure 4 shows the global distribution of crayfish families and areas where *Aphanomyces astaci* is known to occur.

Figure 5 shows the relationship of freshwater crayfish to other species, including some that have been investigated as possible hosts for crayfish plague. Only freshwater crayfish and the mitten crab *Eriocheir chinensis* (Benisch 1940) have been infected with crayfish plague in experimental studies.

In some regions, the natural range of freshwater crayfish is determined by environmental conditions. For example, in England and Wales they are restricted to lime-rich waters (Alderman et al 1984) and in Norway, Sweden and Finland to more southern areas with milder winters (Taugbøl and Skurdal 1993).

Translocation of crayfish from North America and Australia has extended the range of freshwater crayfish to various African and Asian countries (Holdich 2002, Huner 2002, Lewis 2002).

In some parts of Europe, such as Sweden and the United Kingdom, native species have been largely replaced by North American species that are larger and grow more rapidly. For example, native species such as *Astacus astacus* have been displaced by introduced species such as *Pacifastacus leniusculus* (Svärdson et al 1991, Svärdson 1992, Vorburger and Ribi 1999).

In Australia, there are three major groups of crayfish: the small burrowers, the moderately sized burrowers and the smooth crayfish (Mills et al 1994). The species of interest to aquaculture are relatively large, fast growing, smooth crayfish with large tails, including the red claw, the marron and the yabby.

Crayfish are keystone species in the ecology of freshwater habitats. Many species are prey for carnivorous fish. Many are herbivores and scavengers of decomposing plant and animal material, and help maintain the balance of aquatic plants and detritus in water bodies (Marren 1986, Holdich 2002). In some countries where stocks of native crayfish have declined after crayfish plague outbreaks, there have been reports of weed overgrowth in rivers. In some areas, the burrowing activity of introduced crayfish species has caused significant damage to the banks of rivers.

Crayfish have been a luxury food in many European countries for several centuries. Sweden, Finland and to a lesser extent other countries such as the United Kingdom have recreational capture industries. Today, Sweden, Finland and France import crayfish from other parts of Europe and the world. The European species *Astacus astacus* remains the most sought-after and highly priced crayfish in Europe, followed by *Pacifastacus leniusculus* (Ackefors and Lindqvist 1994, Ackefors 1998)

Interest in the aquaculture of edible species of crayfish in Europe has increased as a result of the decline in many wild-capture fisheries following crayfish plague outbreaks, habitat degradation caused by pollution, acidification, clearing and dredging, and increases in the number of predators such as eels and other carnivorous fish (Westman and Westman 1992, Skurdal and Taugbøl 2002).

This has resulted in the introduction of plague-resistant North American species and also some susceptible Australian species to several countries, including Spain, Ecuador, Kenya, China and Brazil (Romero 1997, Huner 2002, Lewis 2002). One of the most common introductions is of the plague-resistant North American species *Pasifastacus leniusculus*, which has a good flavour, fast growth rate and early maturity (Svårdson 1992, Ackefors and Lindqvist 1994, Ackefors 1998). Once these crayfish become established, it is impossible to eradicate crayfish plague if the animals are carriers of *A. astaci*.

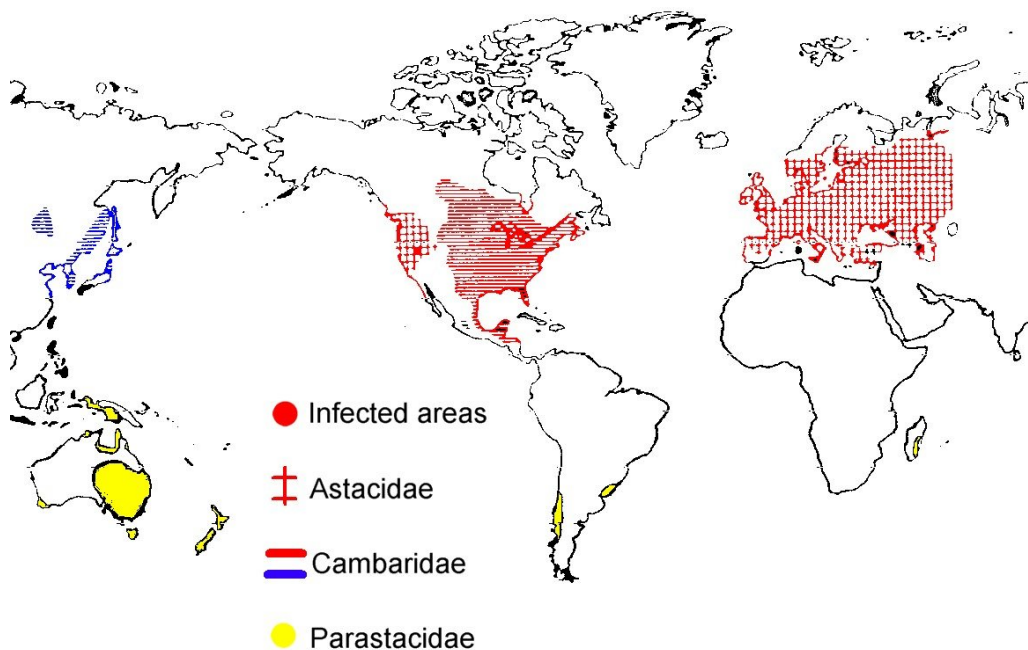


Figure 4 Distribution of crayfish species and *Aphanomyces astaci*

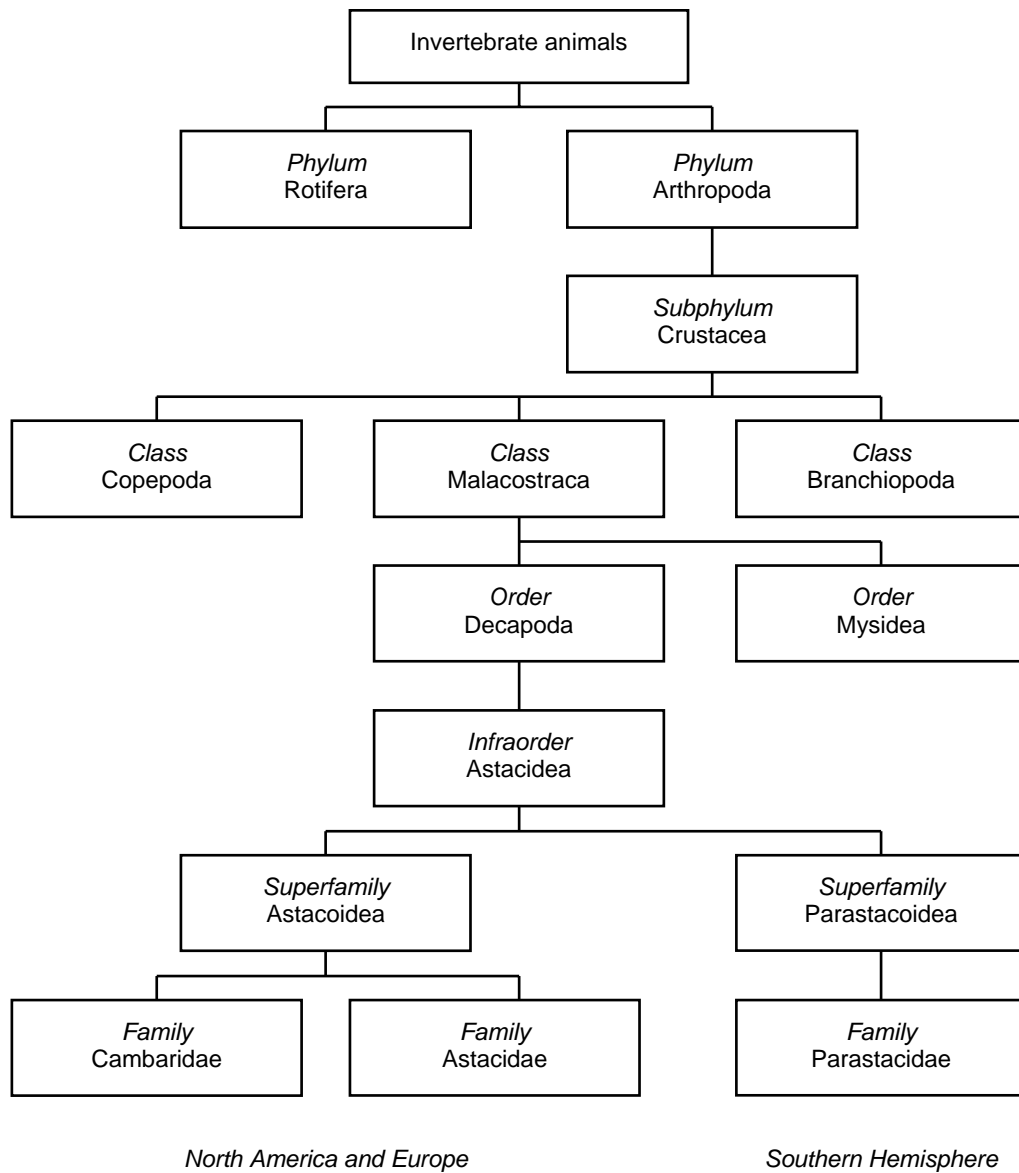


Figure 5 Phylogenetic relationships of the crayfish

Appendix 3 Common and scientific names of crustacean species mentioned in text

Common name	Scientific name
Chinese mitten crab	<i>Eriocheir chinensis</i>
marron	<i>Cherax tenuimanus</i>
red claw crayfish	<i>Cherax quadricarinatus</i>
red swamp crayfish	<i>Procambarus clarkii</i>
signal crayfish	<i>Pacifastacus leniusculus</i>
yabby	<i>Cherax destructor</i>

Appendix 4 Diagnosis of *Aphanomyces astaci*

The following methods are used for the diagnosis and identification of *Aphanomyces astaci*. They are based on the methods recommended in the chapter on crayfish plague in the 2003 edition of the OIE *Manual of Diagnostic Tests for Aquatic Animals*.

Sampling

Samples of moribund animals are preferred for identification of *A. astaci*, but if they are not available, specimens for examination should be transported to the laboratory within 12 hours of the death of the animal. The temperature should not go below 4°C, as freezing will destroy the fungus. Animals may be kept at an appropriate temperature by wrapping them in paper and placing the package in a plastic bag, which is then put on ice in a small esky.

Culture

Fungal culture is carried out on IM (isolation medium) at 16–20°C for 15 days. The medium consists of 1.0 g of yeast extract, 5.0 g of glucose, 10 mg oxolinic acid and 12.0 g agar in 1000 mL of natural river water. One gram of penicillin G is filter sterilised and added after autoclaving the other ingredients (Alderman and Polglase 1986).

Place a 1–2 mm excised piece of abdominal cuticle in the middle of an IM agar plate. Positioning the tissue within a sterile stainless steel washer on the plate will assist in allowing the fungus to grow through the agar and away from the contaminating bacterial growth. Incubate the plate at 16–20 °C for 15 days.

Fungi are normally differentiated based on the morphology of sexual reproductive stages. Because these stages are absent in *A. astaci*, general morphology of the oomycetes is usually sufficient. It is unlikely that any other fungus would cause such a rapid onset of high mortality among crayfish.

Identification

A. astaci grows as a colourless colony within the agar, with no aerial hyphae visible. Some superficial growth may be seen at an incubation temperature of 7°C (Alderman and Polglase 1986).

Hyphae may be examined in lactophenol blue wet preparation under low power using a light microscope. *A. astaci* hyphae are typically non-septate and 7–9 µm in width, but may range from 5 µm to 10 µm (Alderman and Polglase 1986).

Actively growing cultures can be tested for the production of zoospores. Use a cover slip to take a thin slice from the growing edge of the fungal colony. Place it into an empty sterile petri dish. Add sufficient tap water to cover the slice of agar. Leave overnight at 20°C. After 18 hours of incubation, examine the slice of the colony under an inverted microscope. Individual primary spores discharge through the tip of the hyphae. Released spores then round up and encyst to form a mulberry-like cluster. All species of *Aphanomyces* have spore clusters with a similar morphology. The process of release to encystment can take 2–5 minutes (Alderman and Polglase 1986).

Histopathology

Muscle or soft tissue of moribund crayfish can be examined in a wet preparation for hyphae. Melanised areas of cuticle may indicate foci of infection. Thinly smear small pieces of tissue onto a glass slide. Allow to dry and stain with Diff-Quick® or Giemsa stain. Examine under a light microscope.

Histology slides of muscle or cuticle can be stained and examined for the presence of distinctive aseptate, wide hyphae. Appropriate stains are haematoxylin and eosin or Grocott's modification of Gomori stain (DJ Alderman, Senior Microbiologist, Centre for Environment, Fisheries and Aquaculture Sciences, Weymouth, United Kingdom, pers comm, August 1998). The Grocott-Gomori stain has the advantage of clearly distinguishing the black stained hyphae against a green background if fast green FCF is used (Drury and Wallington 1980).

Molecular identification

Molecular diagnostic tests are being developed to detect *A. astaci* by polymerase chain reaction (PCR) and fluorescent in situ hybridisation (FISH). These tests will also differentiate *A. astaci* from *A. invadans*, the causative agent of epizootic ulcerative syndrome in freshwater fishes. Epizootic ulcerative syndrome is present in Australia. The development of molecular diagnostic tests is being done by Fisheries Research and Development Corporation project number 2001/621, and will be reported as an Australian and New Zealand Standard Diagnostic Procedure (ANZSDP). The procedure will be accessible from the website of the Sub-Committee on Animal Health Laboratory Standards.⁵

A PCR-based diagnostic test has been developed in Europe (patent pending).

⁵ See <http://www.scahls.org.au> (Accessed 16 June 2005).

Glossary

Aquatic Animal Health Committee	A committee comprising representatives of the Australian government, Australian state and territory governments, the major aquaculture, wild capture, aquarium and recreational fishing industries and a CSIRO representative. The committee provides advice to Primary Industries Ministerial Council on aquatic animal health matters, focusing on technical issues and regulatory policy. <i>See also</i> Primary Industries Ministerial Council
AQUAVETPLAN	<i>Australian Aquatic Veterinary Emergency Plan.</i> A series of technical response plans that describe the proposed Australian approach to an emergency aquatic animal disease incident. <i>See also</i> AUSVETPLAN
Australian Chief Veterinary Officer	The nominated senior veterinarian in the Australian Government Department of Agriculture, Fisheries and Forestry who manages international animal health commitments and the Australian Government's response to an animal disease outbreak. <i>See also</i> Chief veterinary officer
AUSVETPLAN	<i>Australian Veterinary Emergency Plan.</i> A series of technical response plans that describe the proposed Australian approach to an emergency animal disease incident. The documents provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency-management plans.
Chief veterinary officer (CVO)	The senior veterinarian of the animal health authority in each jurisdiction (national, state or territory) who has responsibility for animal disease control in that jurisdiction. <i>See also</i> Australian Chief Veterinary Officer
Chronic infection	Clinically inapparent or low-grade infection that is transmissible and that may eventually lead to clinical disease.
Compensation	The sum of money paid by government to an owner for stock that are destroyed and property that is compulsorily destroyed because of an emergency animal disease.

Control area	A buffer between the restricted area and areas free of disease. Restrictions on this area will reduce the likelihood of the disease spreading further afield. As the extent of the outbreak is confirmed, the control area may reduce in size. The shape of the area may be modified according to circumstances, eg water flows, catchment limits etc. In most cases, permits will be required to move animals and specified product out of the control area into the free area.
Crayfish byproducts	Products of crayfish origin destined for industrial use (eg crayfish meal).
Crayfish products	Crayfish meat products and products of crayfish origin (eg eggs) for human consumption or use in animal feeding.
Dangerous contact animal	A susceptible animal that has been designated as being exposed to other infected animals or potentially infectious products following tracing and epidemiological investigation.
Dangerous contact premises or area	A defined area that has had a direct, and possibly infectious, contact with an infected premises/area. The type of contact will depend on the agent involved in the outbreak but, for example, may involve animal movements or net/equipment movements.
Declared area	A defined tract of land or water that is subjected to disease control restrictions under emergency animal disease legislation. Types of declared areas include <i>restricted area</i> , <i>control area</i> , <i>infected premises</i> , <i>dangerous contact premises</i> and <i>suspect premises</i> .
Decontamination	Includes all stages of cleaning and <i>disinfection</i> .
Disease agent	A general term for a transmissible organism or other factor that causes an infectious disease.
Disinfectant	A chemical used to destroy disease agents outside a living animal.
Disinfection	The application, after thorough cleansing, of procedures intended to destroy the infectious or parasitic agents of animal diseases, including zoonoses; applies to premises, vehicles and other objects that may have been directly or indirectly contaminated.
Disposal	Sanitary removal of carcasses and other things by burial, burning or some other process so as to prevent the spread of disease.
Emergency animal disease	A disease that is (a) exotic to Australia or (b) a variant of an endemic disease or (c) a serious infectious disease of unknown or uncertain cause or (d) a severe outbreak of a known endemic disease, and that is considered to be of national significance with serious social or trade implications. <i>See also</i> Endemic animal disease, Exotic animal disease

Endemic animal disease	A disease affecting animals (which may include humans) that is known to occur in Australia. <i>See also</i> Emergency animal disease, Exotic animal disease
Enterprise	<i>See</i> Risk enterprise
Epidemiological investigation	An investigation to identify and qualify the risk factors associated with a disease.
Exotic animal disease	A disease affecting animals (which may include humans) that does not normally occur in Australia. <i>See also</i> Emergency animal disease, Endemic animal disease
Free area	An area known to be free of the disease agent.
Infected premises or area	The area in which the disease has been confirmed. Definition of an 'infected area' is more likely to apply to an open system, such as an oceanic lease.
Local disease control centre	An emergency operations centre responsible for the command and control of field operations in a defined area.
Mitigation	Reduction in severity of a disease to reduce its impact.
Monitoring	Routine collection of data for assessing the health status of a population. <i>See also</i> Surveillance
Movement control	Restrictions placed on the movement of fish, people and other things to prevent the spread of disease.
OIE Aquatic Code	<i>OIE International Aquatic Animal Health Code</i> (OIE 2004). Published on the internet at: http://www.oie.int/eng/normes/fcode/a_index.htm (Accessed 11 May 2005). <i>See</i> Appendix 1 for further details
OIE Aquatic Manual	<i>OIE Manual of Diagnostic Tests for Aquatic Animals</i> (OIE 2003). Describes standards for laboratory diagnostic tests and the production and control of biological products (principally vaccines). The current edition is published on the internet at: http://www.oie.int/eng/normes/fmanual/A_summry.htm (Accessed 11 May 2005). <i>See</i> Appendix 1 for further details
Operational procedures	Detailed instructions for carrying out specific disease control activities, such as disposal, destruction, decontamination and valuation.
Owner	Person responsible for a premises (includes an agent of the owner, such as a manager or other controlling officer).

Polymerase chain reaction (PCR)	A method of amplifying and analysing DNA sequences that can be used to detect the presence of virus DNA. See also <i>Reverse transcriptase-PCR (RT-PCR)</i> and <i>Nested RT-PCR</i>
Premises or area	A production site, which may range from an aquarium to an aquaculture lease in the open ocean.
Prevalence	The proportion (or percentage) of animals in a particular population affected by a particular disease (or infection or positive antibody titre) at a given point in time.
Primary Industries Ministerial Council	The council of Australian national, state and territory and New Zealand ministers of agriculture that sets Australian and New Zealand agricultural policy (formerly the Agriculture and Resource Management Council of Australia and New Zealand).
Quarantine	Legal restrictions that limit movement, imposed on a place, animals, vehicles, or other things.
Restricted area	The area around an infected premises (or area), likely to be subject to intense surveillance and movement controls. It is likely to be relatively small. It may include some dangerous contact premises (or area) and some suspect premises (or area), as well as enterprises that are not infected or under suspicion. Movement of potential vectors of disease out of the area will, in general, be prohibited. Movement into the restricted area would only be by permit. Multiple restricted areas may exist within one control area.
Risk enterprise	A defined livestock or related enterprise, which is potentially a major source of infection for many other premises. Includes hatcheries, aquaculture farms, processing plants, packing sheds, fish markets, tourist angling premises, veterinary laboratories, road and rail freight depots and garbage depots.
Sensitivity	The proportion of affected individuals in the tested population that are correctly identified as positive by a diagnostic test (true positive rate). <i>See also Specificity</i>
Sentinel animal	Animal of known health status that is monitored to detect the presence of a specific disease agent.
Serotype	A subgroup of microorganisms identified by the antigens carried (as determined by a serology test).
Specificity	The proportion of nonaffected individuals in the tested population that are correctly identified as negative by a diagnostic test (true negative rate). <i>See also Sensitivity</i>

State or territory disease control headquarters	The emergency operations centre that directs the disease control operations to be undertaken in that state or territory.
Surveillance	A systematic series of investigations of a given population (of fish) to detect the occurrence of disease for control purposes, and which may involve testing samples of a population.
Susceptible animal	Animal that can be infected with a particular disease.
Suspect animal	Animal that may have been exposed to an emergency disease such that its quarantine and intensive surveillance, but not pre-emptive slaughter, is warranted. <i>or</i> Animal not known to have been exposed to a disease agent but showing clinical signs requiring differential diagnosis.
Suspect premises or area	Temporary classification of premises containing suspect fish. After rapid resolution of the status of the suspect animals contained on it, a suspect premises is reclassified either as an infected premises (and appropriate disease-control measures taken) or as free from disease. The reason for the suspicion may vary depending on the agent; however, it may involve clinical signs or increased mortality.
Tracing	The process of locating animals, persons or other items that may be implicated in the spread of disease, so that appropriate action can be taken. Trace-back is the process of tracing the origin of infective <i>Aphanomyces astaci</i> zoospores that caused the crayfish plague outbreak. Trace-forward is the process of tracing the possible spread of <i>A. astaci</i> zoospores from infected animals during an outbreak of crayfish plague.
Vaccination	Inoculation of healthy individuals with weakened or attenuated strains of disease-causing agents in order to provide protection from disease.
Vaccine	Modified strains of disease-causing agents that, when inoculated, stimulate an immune response and provide protection from disease.
Vector	A living organism that transmits an infectious agent from one host to another. A <i>biological</i> vector is one in which the infectious agent must develop or multiply before becoming infective to a recipient host. A <i>mechanical</i> vector is one that transmits an infectious agent from one host to another but is not essential to the life cycle of the agent.
Zoning	The process of defining disease-free and infected areas.

Abbreviations

AAHL	Australian Animal Health Laboratory
ANZSDP	Australian and New Zealand Standard Diagnostic Procedure
aqCCEAD	Aquatic Consultative Committee on Emergency Animal Diseases
AQIS	Australian Quarantine and Inspection Service
AQUAVETPLAN	Australian Aquatic Veterinary Emergency Plan
AUSVETPLAN	Australian Veterinary Emergency Plan
CCEAD	Consultative Committee on Emergency Animal Diseases
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	chief veterinary officer
DAFF	Department of Agriculture, Fisheries and Forestry (Australian Government)
FISH	fluorescent in situ hybridisation
IM	isolation medium
OIE	World Organisation for Animal Health (formerly Office International des Epizooties)
PCR	polymerase chain reaction
proPO	prophenoloxidase

References

- Ackefors H (1998). The culture and capture crayfish fisheries in Europe. *World Aquaculture* 29:18-24, 64-67.
- Ackefors H and Lindqvist OV (1994). Cultivation of freshwater crayfishes in Europe. In: *Freshwater Crayfish Aquaculture in North America, Europe and Australia*, Hunter JV (ed), Haworth Press, New York, 157-216.
- Alderman D (1993). Crayfish plague in Britain, the first twelve years. *Freshwater Crayfish* 9:266-272.
- Alderman DJ (2002). Aphanomycosis of crayfish: crayfish plague. Research and development technical report W2-064. The Centre for Environment, Fisheries and Aquaculture Science, Weymouth, 121.
- Alderman DJ, Holdich D and Reeve I (1990). Signal crayfish as vectors in crayfish plague in Britain. *Aquaculture* 86:3-6.
- Alderman DJ and Polglase JL (1985). Disinfection for crayfish plague. *Aquaculture and Fisheries Management* 16:203-205.
- Alderman DJ and Polglase J (1986). *Aphanomyces astaci*: isolation and culture. *Journal of Fish Diseases* 9:367-379.
- Alderman DJ, Polglase JL and Frayling M (1987). *Aphanomyces astaci* pathogenicity under laboratory and field conditions. *Journal of Fish Diseases* 10:385-393.
- Alderman DJ, Polglase JL, Frayling M and Hogger J (1984). Crayfish plague in Britain. *Journal of Fish Diseases* 7:401-405.
- Baran I and Soylu E (1989). Crayfish plague in Turkey. *Journal of Fish Diseases* 12:193-197.
- Benisch J (1940). Künstlich hervorgerufener *Aphanomyces* – Befall bei Wollhandkrabben. *Leitschrift für Fischerei* 38:71-80.
- CEFAS (Centre for Environment, Fisheries and Aquaculture Science) (2000). Effects of exposure to high and low temperatures on the survival of the crayfish plague fungus *A. astaci* in vitro and in vivo. Australian Quarantine and Inspection Service, Canberra, 23.
- Cerenius L, Bangyeekhun E, Keyser P, Söderhäll I and Söderhäll K (2003). Host prophenoloxidase expression in freshwater crayfish is linked to increased resistance to the crayfish plague fungus, *Aphanomyces astaci*. *Cellular Microbiology* 5:353-357.
- Cerenius L and Söderhäll K (1985). Repeated zoospore emergence as a possible adaptation to parasitism in *Aphanomyces*. *Experimental Mycology* 9:259-263.

- Cerenius L, Söderhäll K, Persson M and Ajaxon R (1988). The crayfish plague fungus *Aphanomyces astaci* – diagnosis, isolation and pathobiology. *Freshwater Crayfish* 7:131–144.
- Cueller L and Coll M (1983). Epizootiology of the crayfish plague (aphanomycosis) in Spain. *Freshwater Crayfish* 5:545–548.
- Diéguez-Uribeondo J, Huang T-S, Cerenius L and Söderhäll K (1995). Physiological adaptation of an *Aphanomyces astaci* strain isolated from the freshwater crayfish *Procambarus clarkii*. *Mycological Research* 99(5):574–578.
- Diéguez-Uribeondo J and Söderhäll K (1993). *Procambarus clarkii* Girard as a vector for the crayfish plague fungus, *Aphanomyces astaci* Schikora. *Aquaculture and Fisheries Management* 24:761–765.
- Diéguez-Uribeondo J and Söderhäll K (1999). RAPD evidence for the origin of an outbreak of crayfish plague in Spain. *Freshwater Crayfish* 12:313–318.
- Drury RAB and Wallington EA (1980). *Carleton's Histological Technique*, 5th edition. Oxford University Press, Oxford, 406.
- Fürst M (1995). On the recovery of *Astacus astacus* L. populations after an epizootic of the crayfish plague (*Aphanomyces astaci* Shikora). *Freshwater Crayfish* 8:565–576.
- Häll L and Unestam T (1980). The effect of fungicides on survival of the crayfish plague fungus, *Aphanomyces astaci*, Oomycetes, growing on fish scales. *Mycopathologica* 72:131–134.
- Herfort A (2004). *Aquatic Animal Diseases Significant to Australia: Identification Field Guide*. Australian Government Department of Agriculture, Fisheries and Forestry, Canberra.
- Holdich DM (2002). Background and functional morphology. In: *Biology of Freshwater Crayfish*, Holdich DM (ed), Blackwell Science Ltd, Oxford, 3–29.
- Huang T, Cerenius L and Söderhäll K (1994). Analysis of genetic diversity in the crayfish plague fungus, *Aphanomyces astaci*, by random amplification of polymorphic DNA. *Aquaculture* 126:1–10.
- Huner JV (2002). *Procambarus*. In: *Biology of Freshwater Crayfish*, Holdich DM (ed), Blackwell Science Ltd, Oxford, 541–584.
- Lewis SD (2002). *Pacifastacus*. In: *Biology of Freshwater Crayfish*, Holdich DM (ed), Blackwell Science Ltd, Oxford, 511–540.
- Lilley JH, Cerenius L and Söderhäll K (1997). RAPD evidence for the origin of crayfish plague outbreaks in Britain. *Aquaculture* 157:181–185.
- Lilley JH and Inglis V (1997). Comparative effects of various antibiotics, fungicides and disinfectants on *Aphanomyces invaderis* and other saprolegniaceous fungi. *Aquaculture Research* 28:461–469.

- Marren P (1986). The lethal harvest of crayfish plague. *New Scientist*, 30 January, 46–50.
- Mills BJ, Morrissy NM and Huner JV (1994). Cultivation of freshwater crayfish in Australia. In: *Freshwater Crayfish Aquaculture in North America, Europe and Australia*, Huner JV (ed), Haworth Press, New York, 217–289.
- Nybelin O (1931). Undersökningar över kräftpestens orsak. *Ny Svensk Fiskeritidskrift* 15:144–149.
- Nybelin O (1936). Untersuchungen über die Ursache der in Schweden gegenwärtig vorkommenden Krebspest. *Meddelanden från Statens Undersöknings-och Försöksanstalt för Sötvattensfisket* 9:1–32.
- Nyhlén L and Unestam T (1975). Ultrastructure of the penetration of the crayfish integument by the fungus parasite, *Aphanomyces astaci*, Oomycetes. *Journal of Invertebrate Pathology* 26:353–366.
- Nylund V, Kirjavainen J, Tulonen J and Westman K (1993). The spread of crayfish plague (*Aphanomyces astaci*) and its effects on the noble crayfish (*Astacus astacus*) population in the Lake Ormajärvi waterway in Finland in 1988–1991. *Freshwater Crayfish* 9:273–279.
- Nylund V and Westman K (1983). Frequency of visible symptoms of the crayfish plague fungus (*Aphanomyces astaci*) on the American crayfish (*Pacifastacus leniusculus*) in natural populations in Finland. *Freshwater Crayfish* 5:277–283.
- Nylund V and Westman K (1995a). Frequency of visible symptoms of the crayfish plague fungus (*Aphanomyces astaci*) on the signal crayfish (*Pacifastacus leniusculus*) in natural populations in Finland in 1979–1988. *Freshwater Crayfish* 8:577–588.
- Nylund V and Westman K (1995b). The crayfish mortality register as an aid in the control of crayfish diseases in Finland. *Freshwater Crayfish* 10:363–373.
- Oidtmann B, Cerenius L, Schmid I, Hoffman R and Söderhäll K (1999). Crayfish plague epizootics in Germany – classification of two German isolates of the crayfish plague fungus *Aphanomyces astaci* by random amplification of polymorphic DNA. *Diseases of Aquatic Organisms* 35:235–238.
- Oidtmann B, Heitz E, Rogers D and Hoffman RW (2002). Transmission of crayfish plague. *Diseases of Aquatic Organisms* 52:159–167.
- OIE (World Organisation for Animal Health, formerly Office International des Epizooties) (2003). *Manual of Diagnostic Tests for Aquatic Animals*, 4th edition. OIE, Paris.
- OIE (World Organisation for Animal Health, formerly Office International des Epizooties) (2004). *Aquatic Animal Health Code*, 7th edition. OIE, Paris.
- Polglase J and Alderman D (1984). Crayfish plague threatens UK stock. *Fish Farmer* 7:16–17.

- Rahe R and Soylu E (1989). Identification of the pathogenic fungus causing destruction to Turkish crayfish stocks (*Astacus leptodactylus*). *Journal of Invertebrate Pathology* 54:10-15.
- Rantamäki J, Cerenius L and Söderhäll K (1992). Prevention of transmission of the crayfish plague fungus (*Aphanomyces astaci*) to the freshwater crayfish *Astacus astacus* by treatment with MgCl₂. *Aquaculture* 104:11-18.
- Reynolds JD (1988). Crayfish extinctions and crayfish plague in central Ireland. *Biological Conservation* 45:279-285.
- Romero XM (1997). Production of redclaw crayfish in Ecuador. *World Aquaculture* 28:5-10.
- Roy JS (1993). Effects of *Aphanomyces astaci* and *Aeromonas hydrophila* on the Australian red claw crayfish *Cherax quadricarinatus*. Thesis. Department of Fisheries and Allied Aquaculture, Auburn University, Auburn, Alabama, USA, 79.
- Schäperclaus W (1927). Krebssterben und krebskrankheiten in der Mark. *Mitteilungen der Fischeri vereine f.d. Prov. Brandenburg* 19:316-328.
- Schäperclaus W (1935). Die Ursache der pestartigen Krebssterben. *Zeitschrift für Fischerei und deren Hilfswissenschaften* 33:343-366.
- Schikora F (1906). Die Krebspest. *Fischerei-Zeitung* 9:529-532, 561-566, 581-583.
- Skurdal J and Taugbøl T (1992). Crayfish management in Europe. *Finnish Fisheries Research* 14:33-37.
- Skurdal J and Taugbøl T (2002). *Astacus*. In: *Biology of Freshwater Crayfish*, Holdich DM (ed), Blackwell Science Ltd, Oxford, 467-510.
- Smith VJ and Söderhäll K (1986). Crayfish pathology: an overview. *Freshwater Crayfish* 6:199-211.
- Söderhäll K (1981). Fungal cell wall β -1,3-glucans induce clotting and phenoloxidase attachment to foreign surfaces of crayfish haemocyte lysate. *Developmental and Comparative Immunology* 5:565-573.
- Söderhäll K and Cerenius L (1999). The crayfish plague fungus: history and recent advances. *Freshwater Crayfish* 12:11-35.
- Sritunyalucksana K and Söderhäll K (2000). The proPO and clotting system in crustaceans. *Aquaculture* 191:53-69.
- Svärdson G (1992). Ecological co-evolution of the parasitic fungus *Aphanomyces astaci* and its crayfish host. *Finnish Fisheries Research* 14:135-143.
- Svärdson G, Fürst M and Fjälling A (1991). Population resilience of *Pacifasticus leniusculus* in Sweden. *Finnish Fisheries Research* 12:165-177.
- Taugbøl T and Skurdal J (1993). Crayfish plague and management strategies in Norway. *Biological Conservation* 63:75-82.

- Treves-Brown KM (2000). *Applied Fish Pharmacology*. Kluwer Academic Publishers, Dordrecht, The Netherlands, 309.
- Unestam T (1969a). On the adaptation of *Aphanomyces astaci* as a parasite. *Physiologica Plantarum* 22:221–235.
- Unestam T (1969b). Resistance to the crayfish plague in some American, Japanese and European crayfishes. *Report of the Institute of Freshwater Research, Drottningholm* 49:202–208.
- Unestam T (1972). On the host range and origin of the crayfish plague fungus. *Report of the Institute of Freshwater Research, Drottningholm* 52:192–198.
- Unestam T (1973). Fungal diseases of crustacea. *Review of Medical and Veterinary Mycology* 8:1–20.
- Unestam T (1975). Defence reactions in and susceptibility of Australian and New Guinean freshwater crayfish to European-crayfish-plague fungus. *Australian Journal of Experimental Biology and Medical Science* 53:349–359.
- Unestam T and Söderhäll K (1977). Specialization in crayfish defence and fungal aggressiveness upon crayfish plague infection. *Freshwater Crayfish* 3:321–331.
- Unestam T and Weiss DW (1970). The host–parasite relationship between freshwater crayfish and the crayfish disease fungus *Aphanomyces astaci*: responses to infection by a susceptible and a resistant species. *Journal of General Microbiology* 60:77–90.
- Vorburger C and Ribi G (1999). Aggression and competition for shelter between a native and an introduced crayfish in Europe. *Freshwater Biology* 42:111–119.
- Westman K (1991). The crayfish fishery in Finland – its past, present and future. *Finnish Fisheries Research* 12:187–216.
- Westman K (1992). Management of the noble crayfish *Astacus astacus* (L.) and the signal crayfish *Pacifastacus leniusculus* (Dana) in Finland. *Finnish Fisheries Research* 14:39–51.
- Westman K and Westman P (1992). Present status of crayfish management in Europe. *Finnish Fisheries Research* 14:1–22.

Index

- abbreviations, 55
- aetiology, 9
- Aphanomyces astaci*
 - likelihood of introduction, 21
 - modes of transmission, 20–21
 - reservoirs, 17–18
 - sources, 17
- Australian Animal Health Laboratory
 - diagnostic tests, 15
- Australian crayfish production systems, 24
- clinical signs of disease, 13
- compensation. *See* cost sharing
- control and eradication, 23–32
 - control and containment, 30–31
 - decontamination, 27–28, 36
 - destruction of crayfish, 26, 35
 - emergency harvesting, 31–32
 - epidemiological investigations, 34
 - eradication, 29–30
 - feasibility in Australia, 29–32
 - international experience, 23
 - policy, 33–37
 - public awareness, 29
 - quarantine, 25, 35
 - response options, 34
 - restocking, 29, 36
 - sentinel animals, 29, 36
 - social and economic effects, 36
 - strategies, 34
 - surveillance, 26, 36
 - tracing, 26, 36
 - trade considerations, 32
 - treatment of products, 27, 35
 - vectors, 28
 - zoning, 26
- cost sharing, 37
- crayfish products
 - treatment, 27, 35
- decontamination, 27–28, 36
- destruction of crayfish, 26, 35
- diagnosis, 12–16, 47–48
 - clinical signs, 13
 - differential, 15
 - in the laboratory, 15
 - laboratory tests, 15
 - pathology, 13–14
- differential diagnosis, 15
- disposal, 27, 35
- emergency harvesting, 31–32
- epidemiological investigations, 34
- epidemiology, 17–21
- eradication. *See* control and eradication
- feasibility of control in Australia, 29–32
- funding. *See* cost sharing
- glossary of terms, 49
- histopathology, 14
- immunity, 16
- laboratory diagnosis, 15
- laboratory tests, 15
 - submission of specimens, 15
- movement controls. *See* quarantine
- occurrence in Australia, 11
- OIE Disease Technical Card, 39
- OIE International Aquatic Animal Health Code, 39
 - zoning, 26
- OIE Manual of Diagnostic Tests for Aquatic Animals, 39
- pathology, 13–14
 - gross lesions, 13
 - histopathology, 14
- predisposing factors, 18–20
- proof of freedom, 37
- public awareness, 29
- quarantine, 25, 35
- references, 57
- resistance. *See* immunity
- restocking, 29
- sentinel animals, 29, 36
- social and economic effects, 36
- submission of specimens, 15
- surveillance, 26, 36
- susceptible species, 9–11
- tracing, 26, 36
- transmission of *Aphanomyces astaci*, 20–21
- treatment
 - crayfish products, 27, 35
- vectors, 28
- world distribution, 11
- zoning, 26, 30–31, 34, 35